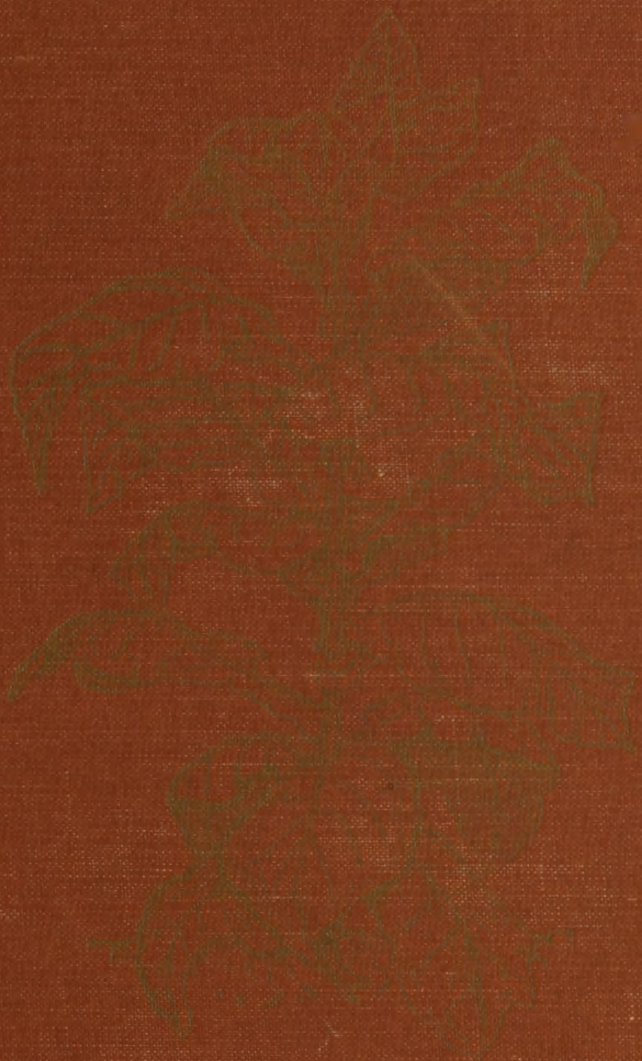

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TOBACCO
DISEASES AND DECAYS

Jean Nicot (1530-1600), in whose honor the tobacco plant was named. In 1561, while ambassador to Portugal, he sent tobacco seed to Catherine di Medici.— *From a picture by Golzius engraved by Pye.*

The first illustration of the tobacco plant to appear in an English publication. — *From Nicholas Monardes's* Joyfulle Newes out of the Newe Founde Worlde, *translated from the Spanish by John Frampton in 1557.*

TOBACCO
DISEASES AND DECAYS

By

FREDERICK A. WOLF

Professor of Botany, Duke University

DUKE UNIVERSITY PRESS

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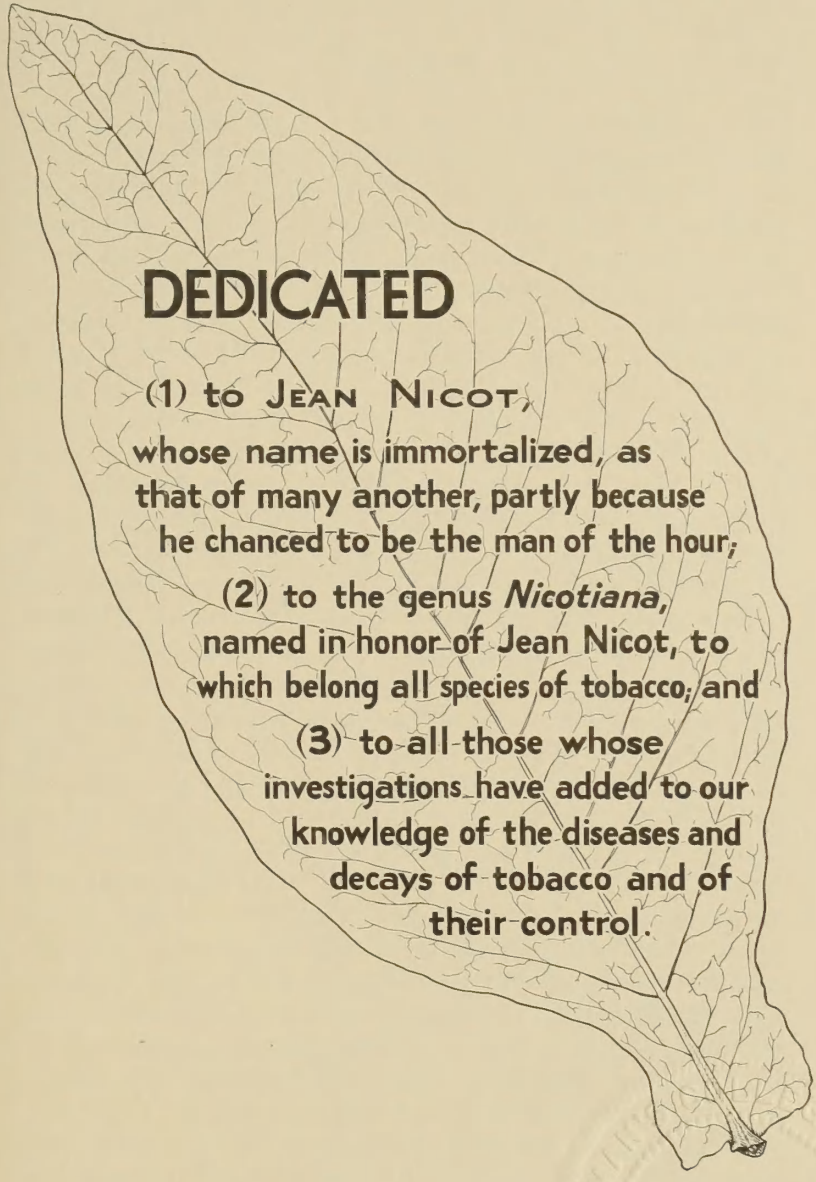

DEDICATED

(1) to JEAN NICOT,

whose name is immortalized, as that of many another, partly because he chanced to be the man of the hour;

(2) to the genus *Nicotiana*, named in honor of Jean Nicot, to which belong all species of tobacco; and

(3) to all those whose investigations have added to our knowledge of the diseases and decays of tobacco and of their control.

DUKE UNIVERSITY

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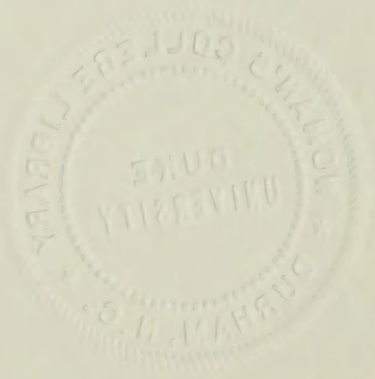

Preface

THE AIM of this volume is to present a discussion of the present knowledge of the diseases of the tobacco plant occurring in all parts of the world where this crop is cultivated, and of the decays of tobacco after harvest. The need for such a publication becomes apparent when one realizes that tobacco is cultivated intensively in many portions of the North Temperate, Torrid, and South Temperate Zones, and that people generally are interested in tobacco.

It is hoped that this book may serve as a guide to growers in the identification of tobacco diseases and in the application of preventive, palliative, and control measures, and as a source of information to agricultural advisers, to whom much of the published material on the diseases of tobacco is inaccessible and unavailable, and to students and investigators who are desirous of learning the present status of our knowledge of any particular disease and of pursuing further inquiry into any special problem. Investigators beginning a study of tobacco diseases should find this volume of value in orienting themselves in the study of their particular problem. The reader will most assuredly become aware of the fact that research on tobacco diseases is abundantly needed, even though many fundamental facts have already been established. Should this book fail in all of the purposes mentioned above, but should it stimulate further investigation of any of the diseases and decays of the tobacco plant, even then its publication might be justifiable.

The author has attempted to bring together in this volume the scattered literature, published in many languages, on the diseases and decays of tobacco. Omissions most certainly have been made from the list of references assembled at the conclusion of this account. These publications include popular accounts, compilations, and technical reports of results of research by investigators in

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all parts of the world. No effort has been made to classify them into these categories nor to evaluate them, an undertaking that I do not feel qualified to perform even though I have been deeply interested in the problem of the diseases of tobacco for nearly twenty years.

A uniform plan of discussion of each disease has not been followed because of the lack, in many cases, of experimentation and, consequently, lack of information. The urgent need for experimentation on control of many of the diseases (this work to be conducted in the field and not in the laboratory or in the greenhouse) is all too apparent to any one who attempts to aid growers in their tobacco disease problems. In general, an attempt has been made to describe the symptoms of each disease, then to consider the cause of each one and the conditions which favor its development and dissemination, and finally to discuss palliative and remedial measures. Citation to pertinent literature is made with each disease, and such publications may contain references to other papers, as important, perhaps, as those cited in connection with any specific disorder.

The completion of this volume has been made possible largely through the aid of a number of colleagues and cooperative agencies. I am under a lasting debt of gratitude for the continued, whole-hearted coöperation, throughout a period of years, of W. W. Garner, Principal Physiologist in Charge, Office of Tobacco and Plant Nutrition, United States Department of Agriculture, and of E. G. Moss, Assistant Director, Tobacco Experiment Station, Oxford, N. C. I am also appreciative of the courtesies extended by James Johnson, W. D. Valleau, W. B. Tisdale, S. A. Wingard, J. E. McMurtrey, Jr., S. C. J. Jochems, K. Nakata, and S. G. Lehman, my colleagues in research on diseases of the tobacco plant, in connection with the loan of illustrations for which credit is acknowledged. Some of the experimental results included herein are from unpublished work by my former associates, G. A. Brecher, John Ehrlich, and L. B. Wilson. Members of the staffs of the Libraries of the United

States Department of Agriculture, the Library of Congress, and the Libraries of Harvard University have generously given of their time and have helped in other ways in making available for my perusal the literature on tobacco diseases, for which I extend grateful acknowledgment. The Board of Governors of Harvard University granted me a research fellowship for the academic year 1933-1934, and thus made available to me the facilities of the Biological Laboratories, for which privileges I express my sincerest thanks. In addition, certain photographs were made for me by Frank White, technical assistant in the Department of Botany at Harvard University, to whom I acknowledge my gratefulness. I have also had the hearty coöperation of the Staff of the Tobacco Research Laboratories that has been a very valuable aid in the completion of this work. Whatever merit this volume may possess is due in generous measure to the coöperation given by my colleagues and these several agencies; whatever its deficiencies, I am compelled to assume full responsibility.

Frederick A. Wolf.

Duke University
Durham, N. C.
January, 1935.

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TOBACCO
DISEASES AND DECAYS

I

Introduction

Any consideration of the diseases of a particular kind of plant is of limited value, and it is particularly true in the case of tobacco, because so much of our information regarding its diseases has come from researches conducted during recent years. Among the earliest attempts to make a comprehensive study of the diseases of tobacco is that of Delacroix. In speaking of mosaic, one of the most widely known tobacco diseases, he said: "*La connaissance que nous possédons de ces maladies est-elle necessairement incomplete; et leur étude est une des questions les plus compliquées de la pathologie du tabac.*"[1] Even though mosaic has been subjected to more investigation than any other disease of tobacco, this conclusion still remains entirely correct. It becomes apparent furthermore, as one attempts to summarize our knowledge of any particular disease of tobacco, that Delacroix's modest conclusion, if uttered today, could very well have stated that our knowledge of each and every disease of tobacco is incomplete.

Reference

Delacroix, G., Recherches sur quelques maladies du tabac en France. Ann. Inst. Nat. Agron. Ser. 2, **5**: 141-232. 1906.

Nature of Plant Diseases

A proper understanding of the concept included in the connotation "diseases of plants" can best be had if this term is defined and briefly discussed. "The composite of reactions of the plant to the causal factor or factors operating upon it" (Whetzel, 1929) defines what is meant

[1] "The knowledge that we possess of these diseases is necessarily incomplete, and their study is one of the most baffling among the disorders of tobacco."

by diseases in plants. This causal factor, whether it be living or non-living, must be capable of inducing abnormal physiological reactions in the plant. These reactions are manifest by some modification, either a stimulation, an inhibition, or an interruption of the normal physiological activities of the plant, and are called symptoms. These reactions may eventually lead to the formation of abnormal structures, to the death of any of the plant organs or parts of organs, or even to the premature death of the individual. Living plants are, therefore, subject to disease, but the decays of dead plants, produced by microörganisms, are not to be regarded as diseases. Sometimes the same species of microörganisms that cause the death of plants or of their parts can also cause decay. In this work, information bearing upon certain decays of the tobacco crop, after it has been harvested, is arbitrarily assembled in a separate chapter.

Any organism that is capable of inducing disease is called a pathogen. The plant that reacts, as the result of inoculation with the pathogen, is the suscept. Many diseases are not primarily caused by organisms, as is the case with those due to unfavorable temperatures, to improper water relations, to a deficiency of mineral elements used in nutrition, or to the presence of poisonous gases.

In general, plant diseases are of three types: (1) those that are manifest by a killing of the cells—necrosis, (2) those that are manifest by a development increased beyond the normal—hyperplasia, and (3) those that are manifest by a development decreased from the normal—hypoplasia. Further classification may then be made within each of these three groups.

REFERENCE

WHETZEL, H. H., The terminology of phytopathology. INTERNAT. CONGRESS OF PLANT SCIENCES. Ithaca, N. Y. **2**: 1204-1215. 1929.

KINDS OF DISEASES OF THE TOBACCO PLANT BASED UPON CAUSES

Too little is yet known of the diseases of the tobacco plant to permit of their classification into an operable sys-

tem that is based upon the symptoms produced, either morphologic or histologic. For convenience, therefore, it is deemed best at this time to group together all those disorders whose causes are related. This logically leads to their tentative separation into (1) those caused by living organisms, (2) those whose causes are non-living, and (3) those caused by viruses. The first includes the bacterial diseases of tobacco, the diseases caused by fungi, the diseases caused by nematodes, and the diseases caused by seed plants. The second includes the diseases caused by the mineral elements when present in excessive or insufficient amounts, those disorders caused by unfavorable water relations, and other diseases of non-living cause. Many investigators regard the viruses as animate, but the evidence for the establishment of whether they are living or not is as yet insufficient.

Classification of Tobacco

Under the name *tobacco* are included all species and varieties of the genus *Nicotiana*. There is no accord among botanists as to the number of species in this genus. Some consider that there are approximately one hundred species, and others that there are approximately forty species but with fifty additional subspecies and varieties. Of these, only two species, *Nicotiana tabacum* L. and *Nicotiana rustica* L., are cultivated commercially. The other species mentioned in this publication are of importance only in so far as they are subject to the same diseases that affect the commercial species and varieties.

It seems desirable also to mention that there is a taxonomic relationship of tobacco to such other plants as potato, tomato, pepper, eggplant, petunia, Jimson weeds, nightshade, horse nettle, and ground cherries. All of these plants, as is well known to those familiar with botanical classification, belong to one and the same family, *Solanaceae*. This relationship accounts for the occurrence of similar diseases of tobacco on these other species.

History of Tobacco

Much has been written about the history of tobacco, but historians do not agree on such features as are concerned with the origin of the name, the early introduction of tobacco into Europe, and its early uses. They seem to agree, however, that the original home of the tobacco plant is America. Only two species, *Nicotiana suaveolens* and *N. fragrans*, are undoubtedly of extra-American origin. These are native of Australia. *Nicotiana tabacum*, varieties of which comprise the greater part of the tobacco of commerce, is native of the West Indies, Mexico, northern South America, and Brazil. *Nicotiana rustica* is grown commercially in Turkey, Russia, and adjacent regions, and originated in Mexico and the contiguous portions of the southern United States.

There is every reason to believe that tobacco was used in America in prehistoric times, as evidenced by the finding of wonderfully wrought pipes among the early Indian tombs in Mexico. The Indians most certainly employed it for a variety of medicinal purposes, and smoked it in ceremonial rites at a period long before the advent of the white man to the Western Hemisphere.

The fact is often lost sight of that the most important and most familiar date in the history of America, October 12, 1492, should be remembered for reasons other than that Columbus discovered America on that date. One historian has said that when the watchman on the Pinta, on the evening of October 11, 1492, sang out "Land ho!" he said something, for this date marks the discovery in addition of a new archipelago, the West Indies, a new western trade route, a new race of people, the Indians, and last but not least, a new plant, the tobacco plant, which its devotees regard as a blessing to humanity.

Columbus first observed the use of tobacco by the Indians while he was exploring the island Guanahani (the Indian name for San Salvador). They rolled the dried leaves of a certain plant and placed them in a hollow cane or forked stick. The leaves were then ignited, one end of the stick was placed to the nostrils or between the lips,

and, by inhaling and puffing, clouds of smoke were emitted from the nostrils and mouth. The Indians called this stick or cane instrument *tabago*. There is no authentic record of the name that they applied to the plant whose leaves they smoked. European explorers gave the name of the instrument to the plant, so that the name *tabago*, then used, soon became *tabaco* in Spanish and *tobacco* in English. There is good reason for believing that the name given to tobacco has no relation to the name of the island Tabago, another of the West Indian Islands, situated near the mainland of South America.

Considerable confusion exists as to the date when tobacco was first cultivated in Europe. Such explorers as Ramon Pane, who accompanied Columbus on his second voyage to the New World, Fernandez Cortez (1515), and Oviedo (1525), on returning, brought seed to Europe. It seems certain that it was first grown in Europe because of its reputed medicinal properties. Concerning its medicinal value, Nicholas Monardes, a physician of Seville, Spain, says:

It is of greate efficacie, notwithstandyng one may make thereof an oyntment, which is singuler to cleanse, incarnate, and knit together all maner of woundes. It hath vertue to heat and dissolve with some bindyng and comfortyng, it gleweth together and doeth soder the freshe woundes, and doeth heale them, the filthy woundes and soares it doeth cleanse and reduce them to perfite health. . . . Tobacco hath particular vertue to heale griefes of the head and . . . griefe of the stomake. . . . In wormes, and in all kinde of them, it killeth them, and doeth expell them marveilouslie. . . . In the tooth ache, putting to it a little baull made of the leafe of the tabaco, it taketh awaie the paine. . . . When thei use to travaile by waies where thei finde no water nor meate: Thei take a little baull of these, and put it betwene the lower lippe and the teethe, and thei goe chewyng it all the tyme that thei doe travell, and that whiche thei doe chewe thei doe swallowe it doune, and in this sorte thei doe journey three or fower daies without havyng neede of meate or drinke, for thei feele no hunger, drieth nor weakenesse nor travaile doeth trouble them.[2]

[2] From John Frampton's translation of Nicholas Monardes's *Joyfulle Newes out of the Newe Founde Worlde* (1577).

André Thevet, in 1555, introduced tobacco into France on his return from Brazil, where the aborigines, he found, called it *petun*. The honor of introducing tobacco into France is usually accorded to Jean Nicot, French ambassador to Portugal, who sent seed from the Royal Gardens in Portugal to Catherine di Medici, the queen of France. Thevet is recorded to have planted seed and cultivated the plants near Angoulême, France, his native town, six years prior to the sending of seed by Jean Nicot.

The account of Thevet's observations on this trip to Brazil, *Singularites de la France Antarctique*, that was translated into English by some unknown person in 1568, contains the first botanical description of the tobacco plant. The first illustration of it to appear in an English book was that in John Frampton's *Joyfulle Newes out of the Newe Founde Worlde* (1577), a translation of Nicholas Monardes's account of medicinal plants obtained from the West Indies.

General Observations

To one with a limited experience with the diseases of tobacco, this volume may at first tend to be a source of discouragement and confusion because of the large number of diseases discussed. It must be remembered, however, that not all are diseases of major importance in any locality. A striking similarity of kinds of tobacco diseases is apparent wherever this crop is grown, but differences in climate of two countries or localities may cause a given disease to be of major importance in one section and of minor importance in another. Seasonal differences in any one locality, moreover, may result in the occurrence of a disease in destructive proportions one year, and may result in no appreciable damage in the following year.

The similarity in kinds of tobacco diseases in all countries may be accounted for in two ways. In the first place, many tobacco diseases are definitely known to be seed-borne, and the evidence in the case of others strongly suggests transmission of the pathogen with the

seed. Seed-borne diseases could be expected to become widely disseminated throughout the world. In the second place, infection in a goodly number of diseases occurs as the result of planting tobacco in infested soils. Some of these soil-borne organisms are pathogenic to species other than tobacco. The organisms responsible for those diseases that are soil-borne and that are not restricted to the tobacco plant, may have parasitized other species for years prior to the employment of these soils in tobacco culture. It is quite apparent to one acquainted with the causes of the different diseases to which tobacco is subject that relatively few of the diseases, caused by specific pathogens, occurring on tobacco, are restricted to the tobacco plant. This does not appear to be true with diseases of other crops to the extent that it is known to be the case with tobacco. The writer feels that so far as the multiplicity of disorders and specific diseases is concerned, the tobacco plant is to be regarded as another victim of civilization. In its wild state, apart from the conditions imposed by artificial culture, tobacco probably was relatively free from diseases.

II

The Seed Bed as Related to the Problem of the Control of Tobacco Diseases

General Considerations

A variety of practices obtain in different tobacco-growing districts as to the types of construction and methods of management of the seed bed. These practices have been devised to promote and stimulate the growth of the seedlings. The control of pathogenic fungi has been a secondary consideration. Many of these practices have come into being as the result of long years of experience by growers. No doubt some of them could not be modified with benefit even were they based upon the results of extensive scientific experimentation. It is readily apparent, however, that the production of healthy tobacco seedlings has not hitherto been sufficiently emphasized as one of the most essential operations in the successful culture of tobacco. The writer believes that this fact can not be overemphasized, for if fields must be planted with diseased seedlings the chances of producing a profitable crop are thereby greatly reduced.

Apparently no thoroughgoing investigation, extending over a period of years, has been made of soil-borne and seed-borne organisms that may attack tobacco seedlings. Investigations have disclosed that a considerable number of the tobacco diseases that are most destructive to the growing crop have their origin in the seed bed. The successful prevention and control of these diseases should, therefore, be expected to require the employment of sanitary measures in connection with the preparation and care of the seed beds. Everyone interested in the culture of tobacco should understand the principles of seed-bed sanita-

tion and the value of their employment in connection with the growing of healthy tobacco seedlings. While a knowledge of these principles and of their application does not constitute a guarantee of success in the control of tobacco diseases, because not all tobacco diseases have their origin in the seed beds, yet seed-bed sanitation constitutes an efficient and economical aid in the control of such diseases as black root rot, sore-shin, wildfire, angular leaf spot, frog eye, *Alternaria* leaf spot, and mosaic.

Seed-bed sanitation as a means of control of specific diseases is discussed in subsequent chapters. Its importance in tobacco culture is destined to be more and more appreciated by growers. It is believed that a special treatment of the subject is warranted, and for this reason the following brief summary has been prepared.

Seed-Bed Sanitation

The combined experience of all who have given attention to seed-bed sanitation indicates that account must be taken of the following matters:

1. The Soil.—The soil in which tobacco seed is to be sown should be freed from organisms that are pathogenic to tobacco. This can be accomplished by the use of steam, dry heat, or chemicals. Steam sterilization is especially adapted to locations in which the same site for seed beds is employed year after year. The apparatus necessary consists of a steam boiler, a pan, and connecting hose or pipes. The boiler should have a capacity sufficient to furnish a continuous flow of steam to the pan at a minimum pressure of 80 pounds. The pans of galvanized iron should be 9 to 12 or even 16 feet long, 6 to 12 feet wide, and 8 inches deep. Pans of suitable size can be handily moved. The edges of the pan should be sufficiently sharp so that it can be forced down into the soil. Steaming should be continued for thirty minutes.

Sterilization by dry heat is usually accomplished by burning wood and brush upon the sites where the seed beds are to be located. In sections where wood is used as fuel in flue-curing, the small trees, crowns, and small

branches left from the cutting of the wood provides the material to be burned on the seed-bed sites. In some places the surface layer of soil is roasted in iron pans and then spread over the situation in which the seed is to be sown. Soil that has been sterilized by steam or dry heat is more loose, friable, and porous than unsterilized soil, and possesses an additional advantage in that it remains relatively free from weeds and grass, the seeds of which have been killed in sterilization.

Formaldehyde is one of the chemicals commonly employed for soil sterilization. Organic mercury compounds and copper salts have also been employed for this purpose. Formaldehyde should be diluted in the proportion of 1 part to 50 parts of water, and sprinkled over the loose soil in sufficient amount to soak it to a depth of at least 4 inches. This should necessitate the use of 1 gallon per 2 to 3 square feet of soil. Several days are required for the formaldehyde to evaporate before the seed may be sown.

When soils cannot be sterilized by any of these meth-

FIG. 1. Tobacco seed bed typical of those in the flue-cured-tobacco section of North Carolina.

ods, another expedient may be employed. Forest soils are least liable to be contaminated with organisms pathogenic to tobacco, and they contain relatively less weed seed than cultivated soils. For these reasons the beds may be located in forest soils. In order to avoid weed seed, the surface layer of soil should be removed and discarded. The practice of clearing new land each year for seed beds, as has been so generally employed in the past in the flue-cured districts, is not to be recommended because it leads eventually to the destruction of an essential natural resource, the forest.

2. The Site.—Consideration should be given to the site that the seed beds are to occupy because soil sterilization is useless if the beds are so located that they can again become contaminated from near-by infested fields or old seed beds. It is preferable to place them in new locations each year unless the soil has been steam sterilized. The selection of new sites each year is of primary importance in the prevention of downy mildew. In addition, they should be sufficiently distant from the curing barns to avoid possible contamination with tobacco refuse. Seed-bed soils should be well drained, either by virtue of slope or of the construction of suitable ditches. Wet soils are conducive to the development of black root rot, black shank, sore-shin, diseases due to species of *Pythium* and *Olpidium*, and several of the diseases of bacterial origin.

3. The Frames and Covers.—When poles are used for frames, they are seldom if ever used for this purpose in the following year; when boards are used, they may serve for several seasons. The cost of the boards may constitute a sufficient reason for using them as frames for several years. It becomes necessary to sterilize the boards where this practice obtains, by wetting them thoroughly with 1 to 50 formaldehyde solution. It may be advisable instead to use new lumber for frames each season.

While it has not been possible thus far to isolate specific pathogenic organisms from the seed-bed covers,

there is abundant observational evidence that they constitute a source of inoculum for seed beds. If old covers are used, therefore, they should be sterilized, preferably by boiling. Immersion in solutions of disinfectants will also accomplish the same purpose. In this case the covers should be dried before being used, in order for the disinfectant to evaporate or volatilize. The employment of new cloth each season is desirable.

4. Tobacco Refuse or Trash.—Tobacco trash is known to harbor the mosaic virus, the wildfire, and the angular leaf-spot bacteria, the black shank fungus, and several of the leaf-spot fungi that appear on seedlings. It is for this reason that tobacco stems and the waste from curing and grading should not be used as fertilizer for seed beds, or as a repellent for insects on tobacco seedlings. This refuse is a valuable manurial material, but should be applied to crops not related to tobacco.

5. The Seed.—It is essential that seed be produced by plants that are free from disease, because a considerable number of the diseases that appear upon the stem and leaves of the tobacco plant are seed-borne. When seed are known to be produced by healthy plants, seed disinfection is not necessary; otherwise it is an essential operation. As a preliminary to seed disinfection the seed should be freed from fragments of pods, calyx, and leaves by passage through a cleaner, which, by means of air currents, removes all of these materials.

Disinfection can be accomplished by means of a variety of chemicals. Silver nitrate, formaldehyde, bichloride of mercury, and many commercial compounds have been used with satisfactory results. The procedure is quite the same, no matter what chemical is employed. The silver nitrate solution should be made with 1 part of silver nitrate to 1000 parts of water. One tablespoonful of formaldehyde to one pint of water makes a satisfactory formaldehyde solution. The seed to be treated should be placed in cloth bags, and then allowed to remain in the disinfecting solution for five to ten minutes. The bags of seed should immediately afterward be

FIG. 2. Satisfactory equipment for sterilization of seed-bed soils with steam, and essential when permanent seed-bed sites are employed. (From James Johnson.)

FIG. 3. Growing flue-cured tobacco seed. Field of H. P. Webb, near Durham, N. C. Flower stalks are bagged to prevent cross-pollination.

washed in several changes of water. The seed should then be spread out under conditions that will permit them to dry promptly and thoroughly.

Seed disinfection results in the destruction of those organisms that adhere to the surface of the seed. If the seed are infected, however, that is, if the pathogens are within the seed, seed treatment will not be 100 per cent effective. Seed that are two or three years old are least liable to harbor pathogenic forms. Seed retain their viability longer than do the pathogens within or on the seed.

6. Fertilizer for Seed Beds.—The soils used in growing tobacco seedlings should be abundantly supplied with available mineral elements. This condition may be fulfilled by the liberal application of thoroughly decomposed manure to the soil and by its thorough incorporation with the soil prior to sowing the seed. It may be necessary to apply phosphoric acid along with the manure. In many areas manure is not available for this purpose, and commercial fertilizers must be utilized. When this is the case, care must be exercised to avoid injury from excessive amounts of chlorides. Relatively larger quantities of fertilizers are applied to seed-bed soils than to equal areas in the field, and for this reason the materials from which the fertilizers are compounded must be relatively free of chlorides. This necessitates the use of high-grade sulphate of potash or sulphate of potash-magnesia, as the source of potash. Fertilizers containing 8 per cent of phosphoric acid, 4 per cent of nitrogen, and 3 per cent of potash are recommended for use in seed beds in the flue-cured-tobacco area.

7. The Care of Seed Beds.—Seed should not be sown too thickly. A tablespoonful of seed to 100 square yards is usually sufficient.[1] One half of this quantity may be

[1] T. Berthold (*Vergleichende morphologische und physiologische Untersuchungen an einigen Tabaksorten.* Inaugural Dissertation. Munich. 58 pp. figs. 14. 1929) determined that each capsule of the American varieties of tobacco contains approximately 3,000 seeds, with a total weight of about 0.3 gram. With these figures as the basis for calculation, there are 285,000 to 300,000 tobacco seeds in an ounce.

sufficient in some districts. Thinly sown beds are advantageous where downy mildew or blue mold occurs.

Experience has shown that attention must be given to the matter of proper watering and ventilation, especially when the seed beds are under glass. Care must also be exercised to guard against the introduction of inoculum into the seed beds through the careless use of tobacco. The inoculum for such diseases as wildfire, angular leaf spot, and mosaic may be introduced with spittle. In the flue-cured-tobacco districts, growers, while weeding, drawing plants for setting, or otherwise caring for the beds, commonly inoculate the seedlings with mosaic virus by spitting.

Promiscuous visiting of diseased seed beds is inadvisable because the germs may cling to the clothing, shoes, and hands as the result of handling affected seedlings, and thus be conveyed to disease-free seed beds subsequently visited.

Seed beds should be frequently inspected, and if any of the seedlings are diseased, the spread of infection may be checked by the prompt removal of the affected plants and the soaking of the infested area with formaldehyde solution, 1 to 25. This procedure will not check downy mildew, but is effective against some of the root rots. In some cases it is advisable to spray in order to check the further spread of the disease. Bordeaux mixture appears to be the most satisfactory spray material for this purpose. It consists of copper sulphate (blue stone) and lime. The best mixture results when 4 pounds of copper sulphate are dissolved and diluted in 25 gallons of water. Then 4 pounds of quicklime or rock lime must be slaked and diluted to make 25 gallons of milk of lime. Equal portions of each of these stock solutions may then be mixed together to make the standard 4-4-50 Bordeaux mixture. If a less concentrated Bordeaux mixture, as a 2-2-50 mixture, is desired, it can be made by first diluting each stock solution by doubling the volume and then mixing equal portions of the two stock solutions.

Saprophytic Fungi in Seed Beds

Various fungi that thrive only on decaying organic matter have been found to occur in seed beds and may occasion minor damage. They accomplish this either by raising up the surface of the soil and thus permitting the seedlings to wither, by crowding or choking out the seedlings as they emerge from the soil, by inhibiting their growth, or by killing them with products of decay. Certain of them, as the "pink molds," occur only when the beds have been "burned," others, as the shaggy mane mushroom, only when the beds have been manured, and others, as the slime molds, when there is an abundance of decaying wood and leaves that have been incorporated in the surface layer of soil. The list of saprophytic fungi that have been collected in seed beds includes, *Pyronema confluens* Tul., *P. omphalodes* (Bull.) Fkl., *Peziza vesiculosa* Bull., *Cyathus olla* (Batsch.) Pers., *Physarum nefroideum* Rostf., *Stemonitis herbatica* Peck, *Coprinus micaceus* Fr., and *Coprinus commatus* Fr.

Pink Molds.—The name *pink mold* is used to designate the pink crust which appears occasionally in patches on the surface of the soil in seed beds. It is favored by poor drainage, shading of the beds, and poor ventilation. When, in addition, the ground has been sterilized by burning brush and wood upon it, pink mold may abound. The two species of fungi, *Pyronema confluens* and *P. omphalodes*, that are responsible for this condition are normal inhabitants of soil and are not parasitic. It is not established that pink mold is directly responsible for injury to tobacco seedlings. Observations indicate that it may reduce the stand of seedlings by interfering with their coming to the surface. Pink mold seems thus to choke out the stand.

Slime Molds.—Two species of slime molds, *Physarum nefroideum* and *Stemonitis herbatica*,[2] may creep up onto

[2] Other species of slime molds should occur in seed beds. In fact, a list prepared by H. Nakamura shows that in Japan *Didymium nigripes* Fr., *D. squamulosum* Fr., *Fuligo septica* Gmel., *F. septica var. candida* Fr., *Physarum cinereum* Pers., *P. gyrosum* Rost., and *Stemonitis ferruginea* Ehrenb. have been collected in tobacco seed beds.

the seedlings in patches and then produce their characteristic powdery reproductive structures.

Shaggy Mane Mushroom.—Either this species of mushroom, *Coprinus commatus,* or *C. micaceus,* can uproot the seedlings as they come to the surface, and on deliquescing cover over the seedlings beneath them with a liquid which, on drying, becomes a black crust. This mushroom appears in beds that have been enriched with stable manure that is insufficiently decomposed.

Bird's Nest Fungus.—The bird's nest fungus, *Cyathus olla,* as the name indicates, has the appearance of small nests. Each is about an eighth of an inch across, and has a cluster of egg-like bodies in the bottom of the nest. Clusters of these nests are usually formed on bits of decayed wood or upon the dung balls from stable manure.

III

Nutritional Diseases

General Considerations

The present-day concept of mineral nutrition of plants dates with de Saussure's epochal book, *Chemical Researches in Plants* (1804), in which he asserted that green plants utilize ten elements; namely, carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, calcium, magnesium, sulphur, and silicon. About a half century later Knop substituted iron for silicon, which he thought to be non-essential. For more than an additional half century it was generally accepted by botanists that these ten elements only are essential for the proper nutrition of green plants. Within the past few years, however, numerous investigations have shown that additional elements are essential for some plants at least. These physiological researches, supported by chemical analyses of the ash of plants grown under natural conditions, show that plants contain at least thirty-five elements. True, no one maintains that all are essential, or that he knows the complete rôle of any single mineral element in plant nutrition. Admittedly, their presence in the ash does not constitute conclusive evidence that they are indispensable to the green plant. They may have been absorbed accidentally, as some maintain.

Again, some elements are present in the ash in minute traces, so that a group of plant physiologists now speak of such elements as stimulatory elements. This basis of separation of stimulatory elements from essential elements, it is believed, will be found to be wholly untenable.

The tobacco plant is extremely sensitive to deficiencies of the common mineral elements or to the presence of small amounts of others. If, for example, plants are

grown in sandy soil, very definite symptoms of toxicity develop when boron at the rate of one pound per surface acre is applied. Again, when less than 10 pounds per acre of magnesia (MgO) are present, very distinctive effects are noticeable in the character of the foliage. Morgan (1929) has called attention to the sensitivity of tobacco as an indicator plant in studying nutritional deficiencies.

Methods of Investigation of Mineral Nutrition Not Wholly Adequate

The great bulk of the extensive investigations which have been made on the rôle of mineral elements in plant nutrition have a distinctively limited value. This statement is based upon the fact that it is difficult to ascribe differences in results of field plot experiments to a single factor. It is only within the past few years that plant physiologists have come to appreciate that the usual experimental procedures are inadequate in studies of nutritional problems. These procedures employ solution cultures of mineral nutrients or quartz cultures supplied with mineral nutrients. One reason for this is the impurity of "chemically pure" chemicals that are being employed. Another is the growing recognition of the influence of the presence of one element on the absorption of another. Another is the influence of pH on the availability of the mineral components of that substratum. Again, whether all plants require the same mineral elements or whether they exercise selective absorption has not been unequivocally determined.

New experimental procedures must be devised if progress is to be made. Fortunately, a number of investigators in recent investigations have employed chemicals whose purity is attested by refined chemical analytical methods and by spectrographic methods. Use is being made also of a method which involves absorption of the compound under investigation through the exposed roots of potted plants. Another method, which depends upon leaf absorption of elements used by plants in small

amounts, is suggested in studies by Williams (1932), and appears to be worthy of trial.

Many of the numerous investigations which conclude that the lack of iron or of manganese is the cause of specific disorders, are open to criticism. The conclusion that disorders are caused by deficiencies is based usually upon the correction of the disorder following the application of iron salts, or of manganese salts to the foliage of the diseased plant or to the soil around the plant. One might just as logically conclude that malaria is due to a lack of quinine! Moreover, iron salts are extremely difficult to free from manganese and vice versa. Admittedly, it is difficult to prove that a particular diseased condition is caused *directly* by lack of a specific element.

Need for Commercial Fertilizers

The application of commercial fertilizers is a general practice wherever tobacco is cultivated. These fertilizers are compounded to contain phosphorus, nitrogen, and potassium, but other elements always occur in the mixtures, notably calcium, magnesium, and sulphur. Analyses of fertilizers show the amount of phosphoric oxide (P_2O_5), nitrogen (NH_3), and potassium oxide (K_2O) that they contain, and in speaking of them, one calls them, for example, a 4-8-6 fertilizer mixture, by which is understood one that contains 4 per cent of NH_3, 8 per cent of P_2O_5, and 6 per cent of K_2O. Analyses of leaf tobacco give an approximation of the mineral requirements of the tobacco plant as shown in the following tabulation.

Table 1

Number of Pounds of Plant Food Material Contained in 1,000 Pounds of Tobacco Leaf

Constituents	N	K_2O	P_2O_5	CaO	MgO	SO_3	MnO	Cl
Maximum amounts[1]	50	90	12	80	46	25	2.5	70
Minimum amounts[1]	10	10	3	12	1.5	6	.10	2
Average amounts (flue-cured leaf)[1]	20	30	8	40	6	15	1.0	
Average amounts (air-cured leaf)[2]		50	8	61.9	12.8	10		11.5
Average amounts[2] (stems)		34	11.2	15	.5	2.6		2.6

[1] Analyses made by the Office of Tobacco and Plant Nutrition, United States Department of Agriculture.

[2] Computed from data by Preissecker, K., *Der Tabakbau und die Ausbildung des Tabaks zum industriellen Rohstoffe*. Wien, 1914.

These maximum and minimum figures cover the range of leaf tobacco generally, but, of course, should be increased by approximately 50 per cent if the mineral requirements of the plant as a whole, including stem and roots, are considered.

It is quite evident that tobacco requires an abundance of readily available minerals. These minerals are deficient in most areas devoted to tobacco culture. These deficiencies are indicated in Table 2, which shows the composition of certain soils, used in tobacco culture, of the southern United States.

TABLE 2

CHEMICAL COMPOSITION OF TOBACCO SOILS OF THE SOUTHERN UNITED STATES[1]

Series and type	Constituents, per cent							
	N	K_2O	P_2O_5	CaO	MgO	SO_3	MnO	SiO_2
Norfolk sand	.05	.08	.10	.19	.01	.08	.03	94.8
Norfolk sandy loam	—	.10	.06	.39	.09	.07	.01	94.5
Norfolk fine sandy loam	.04	.05	.04	.03	Trace	.05	.01	92.9
Orangeburg sand	.06	.08	.04	.08	Trace	.07	.02	96.2
Orangeburg sandy loam	.03	.10	.05	.05	Trace	.07	.05	94.3
Tifton fine sandy loam	.04	.10	.04	.05	Trace	.06	.03	94.1
Ruston fine sandy loam	.05	.12	.05	.06	.01	.04	.02	90.2
Portsmouth fine sandy loam	.05	.06	.05	Trace	Trace	.03	.003	94.9
Coxville fine sandy loam	.08	.08	.04	.05	.01	.05	.01	92.9
Cecil sandy loam	.04	2.31	.03	.16	.28	—	—	—
Durham coarse sandy loam	.03	1.60	.04	.35	.09	—	—	—
Granville coarse sandy loam	.02	1.12	.04	.15	.36	—	—	—

It may be noted that all of these soils have a low content of nitrogen and phosphorus. The Cecil sandy loam has a high potash content. Incidentally, the heaviest tobacco with the most desirable aroma, texture, and combustibility of the flue-cured type is grown on soils of this series. Tobacco of brightest color is grown on soils of the Norfolk series.

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[1] These analyses were made by the Bureau of Chemistry and Soils, United States Department of Agriculture. They are representative of these types.

Phosphorus

Little is known regarding the rôle of phosphorus in the nutrition of the tobacco plant. Growers know from observation that it is essential for the proper ripening of the leaves. If it is not supplied with the commercial fertilizer, usually as phosphoric acid, especially to soils deficient in phosphorus, as those of the southeastern United States, then tobacco crops will be late in maturing. If, in the case of a crop that lacks sufficient phosphorus, harvesting is delayed long enough, however, the cured product is of good quality.

When no phosphorus is combined with the fertilizer mixture applied to tobacco, the plants are slow in starting to grow following transplanting, and the foliage becomes abnormally dark green in color. Eventually such plants will be of normal stature, with leaves that are narrower and younger in appearance than those on

Fig. 4. Pock-like brown leaf spots on leaves of plants that were grown in soils to which no phosphoric acid was added with the fertilizer.

Fig. 5. Flue-cured tobacco plant exhibiting symptoms of potash hunger. The tips and margins of the leaves are rolled downward and the surface of these leaves is roughened. (From U. S. Dept. Agr.)

plants supplied with sufficient phosphorus. In some seasons numerous small, dark brown, sunken spots appear on plants grown with a deficiency of phosphorus. This has been noted at the Tobacco Experiment Station, Oxford, N. C., and is recorded also on Burley tobacco in Kentucky. Roode roest (red rust) in the Dutch East Indies is believed to be caused by a deficiency of phosphorus. In experiments with solution cultures deficient in phosphorus, McMurtrey (1933) noted a similar condition. Spots did not always occur on plants grown in cultures containing no added phosphorus, so that this apparent relationship to a tissue breakdown remains to be clarified.

REFERENCE

McMURTREY, J. E., JR., Distinctive effects of the deficiency of certain essential elements on the growth of tobacco plants in solution cultures. U. S. DEPT. OF AGR. TECH. BUL. **340**: 42 pp. figs. 12. 1933.

POTASSIUM

GENERAL CONSIDERATIONS.—In the economy of the tobacco plant potash aids (1) in maturing the leaves properly and (2) in producing a cured product of good burning qualities. How this is accomplished is not understood. The botanist's usual response to an inquiry regarding the function of potash in plants is that it is connected in some manner with the formation of starch.

Mixed fertilizers for bright tobacco usually contain from 3 to 5 per cent of potash, but much higher proportions have been safely employed. Potassium salts are usually sulphates or chlorides or mixtures of these two. The use of chlorides, in some soils, is limited because of specific effects from an excess of chlorine. This matter is discussed in the paragraphs which deal with the effects of chlorine.

When seedlings are set in soils to which an excess of potassium salts or of mixed concentrated fertilizer has been stirred, there is difficulty in getting a stand, and growth may be slow at first. In soils containing considerable humus, any excess of potash not required by the growing crop remains, and is leached out with difficulty.

In poor sandy soils enough potash to make a good crop cannot be applied prior to transplanting, for which reason it is advisable to apply a portion before transplanting and the remainder as a "side dressing" or "top dressing" to the growing crop.

The general vigor of tobacco is undoubtedly modified by potash. Plants which are liberally supplied exhibit greater freedom from leaf spot diseases than those given a limited supply. This observation is not supported by specific studies of disease producing organisms on tobacco, but is the consensus of opinion of investigators. It agrees, moreover, with the finding of the influence of potash on susceptibility to disease in several other crop plants.

Agronomists regard potassium as the most important single constituent of mixed fertilizers for tobacco because of its influence on the quality of the cured leaf. This opinion is based upon experiments in which comparisons are made between the effects on tobacco of fertilizers, lacking either in N, K, or P that are applied to soils deficient in these elements. Yields are small when N and P are omitted, although the leaves are of good quality; but when K is omitted, the product has a low market value, or it may be wholly unsaleable.

Potash Deficiency or Hunger.—When the leaves of plants that have been topped are broad, dark green, have plenty of body, and are smooth or only slightly contoured between the veins, the supply of potash may be known to be sufficient. When, however, the edges of the leaves curl downwards and inwards toward the midrib, the tips curl under, the leaves have a bronze overcast color, and are puckered between the veins and along the edges, the supply of potash is very deficient. In early stages this disorder is evident by this off color and by the appearance of small yellowish to brownish specks especially near the leaf tips. If the deficiency is slight, the specks may involve the leaf tips only. If it is excessive, yellow specks will appear between the veins and along the leaf edges and will gradually become brown and dry at the cen-

ters. The spots gradually enlarge and fuse to form large, dead areas involving much of the leaf surface. In some cases these dead areas fall out, causing the leaves to be ragged and perforated. The portion of the leaf that remains green continues to grow, giving the rough puckered surface and rim-bound margin. Leaves on a crop that is markedly deficient in potash do not ripen, but become worse the longer they remain on the hill. When they are cured, they are thin, dull, and without body.

McMurtrey (1933) studied potash hunger in tobacco grown in nutrient solutions to which no potash was added, and secured the same symptom-complex as occurs in plants grown in the field. The lower leaves are the first to be involved, and the bud leaves tend to remain normal. Recovery follows when plants that show potash

FIG. 6. Curling, yellowing, and dying of tobacco leaf tissues along the margins and between the veins, resulting from extreme deficiency of potash.

FIG. 7. Deficiency of potash in Maryland tobacco. The tips and margins of the leaves are yellowish-green, and numerous lesions have formed near the tips, a condition similar to that on flue-cured tobacco when the deficiency is slight. (From U. S. Dept. Agr.)

hunger are transferred to solutions containing potassium. All subsequent new growth is normal, and the old leaves recover, save in the necrotic areas.

Prevention.—One should provide adequate supplies of potash for tobacco, keeping in mind that a reasonable excess will do no injury and will be available for subsequent crops. For bright tobacco 40 to 80 pounds of K_2O per acre appears to be adequate. In order to avoid injury from excess of chlorine, to the growing crop or to the burning quality of the smoking tobacco, the mixed fertilizer should not contain in excess of two per cent of chlorides.

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Nitrogen

General Considerations.—Nitrogen is strikingly essential for the proper growth of the tobacco plant. Quality of the cured leaf is greatly influenced by the availability of this element and the proportionality of this element to the other essential ones. This appears to accord with observations on all types of tobacco. It is especially pronounced in the case of flue-cured tobacco and Burley tobacco, which require that the supply of nitrogen be available in diminishing quantity throughout the season so as to promote uninterrupted plant growth and proper ripening. By the time that the crop has matured the available supply of nitrogen should be practically exhausted. Much larger quantities of nitrogen are required for cigar-filler types of tobacco. The nitrogen supply may be controlled in large measure by the exercise of care in choosing sources of nitrogen for the fertilizer mixture. Best results follow the use of mixtures containing readily available inorganic, and slowly available

organic, nutrients. Attention must also be given to the proportion of nitrogen to the other fertilizer constituents and to the rate of application of the mixed fertilizer. The character of the crops grown in fields to be planted with tobacco should also be considered, since the residual nitrogen left from a previous leguminous crop (clover, vetch, cowpeas, soybeans, peanuts, etc.) may make it inadvisable to add nitrogen to the fertilizer mixture.

Excess of Nitrogen.—The leaves of tobacco plants which are supplied with an excess of nitrogen are larger than normal, have coarse ribs or veins, and are abnormally dark green in color. In such leaves the formation of the pigments, whose presence in appropriate proportions is essential to desirable colors in the cured leaf of all different types of tobacco,[1] is apparently modified or even inhibited altogether. In the case of flue-cured tobacco given excessive amounts of nitrogen, the cured leaves are green, red, brown, or at times nearly black. When the nitrogen supply is exhausted in a crop properly supplied with nitrogen, the leaves become pale green at maturity, and, after being cured and stored in the packing house, are lemon to orange in color. Leaves or plants with excessive nitrogen fail to become pale green, no matter how long they are left in the field. Instead of ripening there is a breaking down of tissues, and the formation of large brownish blotches occurs. The same effect follows a too low topping. When the leaf surface has been excessively reduced by too low topping, the leaves remaining on the plant seem to be unable to properly adjust their metabolism.

Prevention.—This disorder is the direct sequel of the application of excessive amounts of nitrogenous fertilizer materials, and the indirect sequel of improper topping practices. In the former case the disease may be

[1] Recent investigations have shown that there are at least four pigments: quercetin, rhamnetin, quercetagetin, and methyl-quercetagetin (tobaccin) present in the cured leaves of tobacco. The color of the leaves is due to the proportionality of these pigments (from information imparted to the writer by Dr. Nierenstein, University of Bristol). See Nierenstein, M. The natural organic tannins. London, J. & A. Churchill. 319 pp. 1934.

avoided by following fertilizer practices which are known to be successful. Account must always be taken of the state of tilth of the soil and the character of preceding crops. If excessive proportions of readily available nitrogen are applied, no injury may result to the crop if the rainfall during the early part of the season is above normal. Under such conditions much nitrogen is lost by leaching. When the correct amount and proportion of nitrogen has been applied for a normal season, damage may occur when all the rains are light and infrequent, until nearly harvest time, and then nitrogen becomes available when the leaves should be ripening.

If the crop has been topped too low, it need not be kept closely suckered, as is the usual practice. Instead, one or two suckers should be allowed to develop to aid in regulating the growth processes in the leaves which are to be harvested.

Nitrogen Hunger or Deficiency of Nitrogen.—Tobacco plants suffering from nitrogen hunger are dwarfed throughout, with a tendency for such plants to hold the rosette form. The stems are slender, and the entire foliage including blade and veins is light green in color. Local necrotic areas never appear to result. As harvest approaches, the leaves become yellow-green, and if they are removed and cured, the color of the product is very desirable but is lacking in body. If they are allowed to remain on the plant, the green color entirely disappears; the leaves are then lemon-yellow and soon become dry and brown.

Studies on the effects of nitrogen hunger on tobacco grown in solution cultures have been made by Mes (1930) and McMurtrey (1933). Both point out the fact that plants lose their normal, fresh green color within a few days when placed in a nutrient solution containing no added nitrogen. The oldest leaves are the first to lose their color, and the bud leaves tend to retain their color. Plants from which nitrogen has been withheld for a considerable period and which are then transferred to solutions supplied with nitrogen recover rapidly and com-

pletely, save for leaves which have dried up (McMurtrey, 1933). When nitrogen is withheld during early stages of growth, the roots tend to attain greater length but to be less branched than those in cultures containing added nitrogen.

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CALCIUM

GENERAL CONSIDERATIONS.—Calcium or lime is usually applied to soils to neutralize the acids present. By doing so conditions are rendered more favorable for bacterial activity, and, as a sequel, the humus portions of the soil are more completely decomposed. In addition, the decomposition of inorganic compounds is facilitated, and thus the mineral elements are made more readily available to the growing crop.

In tobacco culture the application of ground limestone or of burnt lime to the soil has been employed only in recent years. In general, soils used in growing air-cured Burley tobacco are high in calcium. Ordinarily there is sufficient calcium in complete commercial fertilizers, it being added with the superphosphate, to make unnecessary liming of tobacco soils.

In some regions, as in Kentucky, where tobacco is grown in long rotations with other crops, it seems advisable to use moderate applications of lime. In these cases the lime should be applied to the crop that succeeds tobacco. The relationship between lime, legumes, and the fertility of the soil has long been known. Advantage may be taken of this practice in some sections and with some types of tobacco.

EFFECTS OF LIMING.—The investigations and observations on the reaction of the soil most favorable for flue-cured tobacco and for cigar-leaf tobacco in the Connecticut Valley indicates that a pH of 5.0 to 5.6

is optimum for best results. They also show that when soils are rendered more alkaline than this by liming, there is an increase in pounds of tobacco per acre, and that the cured leaf is heavier bodied and brighter in color. The product from a limed soil is, however, more difficult to age properly and is otherwise more undesirable to the manufacturer, so that buyers have learned to discriminate against it.

Liming for tobacco is attendant with another difficulty which is more noticeable to the grower than the effect on the quality of the cured leaf, because liming modifies the soil reaction so that it is a favorable substratum for the development of black root rot, caused by *Thielavia basicola*. This organism thrives best in alkaline soil and is inhibited by acids. Serious damage following liming may occur in fields that have previously grown satisfactory crops of tobacco for years. Observational evidence indicates that in some cases these fields have probably been more or less infested for a period of years, but tobacco escaped injury so long as the soils remained acid.

Lime has been applied to seed-bed soils to stimulate the rapid growth of tobacco seedlings, thus enabling the grower to transplant at an earlier date than otherwise. This procedure is hazardous in soils infested with *Thielavia basicola*. In one season the writer saw 500 to 600 acres of tobacco, grown in one county in North Carolina, which yielded approximately 50 per cent of a normal crop, apparently because the fields were set with seedlings from infested beds. The beds from which the fields were transplanted had been limed. The crops set from unlimed beds sometimes on the same farm or from unlimed beds on adjoining farms were not affected with black root rot.

Deficiency of Lime.—A lack of sufficient calcium has a very pronounced effect upon all parts of the tobacco plant. It is especially manifest in all growing points, since calcium seems to be essential to cell-wall formation in embryonic tissues. Seedlings grown in washed sand, and nourished with solutions of minerals to which no

calcium has been added, tend to retain their rosette form. The plant shown in Figure 8 had been transplanted approximately six weeks. Control plants nourished with a solution containing calcium had grown meanwhile to a height of 16 to 18 inches. The first noticeable effect of calcium hunger is a peculiar hooking downward of the tips of the bud leaves. This is followed by the death of the leaf tips and margins, so that the leaves are misshapen and distorted. Garner and others (1930) noted this symptom in fields at Upper Marlboro, Maryland, under extreme cultural conditions in which all calcium was withheld from the mineral nutrient solution. McMurtrey (1933) found that the terminal bud is killed. Lateral buds may start to unfold in the leaf axils, but will soon exhibit the same symptoms and die. If calcium is removed early in the growth period, the formation of flowers is inhibited entirely; if, after growth is well started, necrotic tips appear on the corolla and calyx, most of the flowers are shed, and the seed pods are abortive.

Prevention.—As previously stated, ordinary commercial fertilizers contain adequate quantities of calcium to meet the nutritional requirements of tobacco. Apparrently the only cases known of the occurrence of calcium deficiency under field conditions are those reported by Garner and others (1930) in fertilizer plot work in Maryland. In these cases highly purified chemicals were employed in compounding the mixed fertilizers. When they applied the equivalent of 36 pounds of calcium per acre as dicalcic phosphate, the plants did not exhibit symptoms. A number of other compounds were added, without success, to accomplish this correction. While this does not prove that the disorder was due to a deficiency of calcium, it shows that a condition recognized as calcium starvation can be prevented by the application of calcium.

The solution of the problem of calcium hunger is complicated by the fact that magnesium may be highly toxic when calcium is not present in sufficient quantity. This

makes it difficult to distinguish satisfactorily between symptoms due to calcium deficiency and symptoms due to a toxicity from magnesium. The antagonism between Ca ions and Mg ions has long been known, but the relation of this fact to calcium deficiency in tobacco needs further investigation.

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SAND DROWN OR MAGNESIUM HUNGER

The name "sand drown" is applied by growers to a peculiar deficiency disease of tobacco which has no relation to a true drowning, as may result from heavy rainfall or the accumulation and standing of water in low situations or above terraces. Sand drown is aggravated by excessive rainfall, but the effects are primarily those of leaching rather than of the presence of the water itself. Neither is it caused by sand, although it is most severe on deep, sandy soils which may never become sodden or water logged. The name is therefore somewhat misleading, but because of its widespread popular usage it is retained.

SUSCEPTS.—The disease has been investigated primarily as it occurs on tobacco. When soils on which sand drown normally occurs are cropped with cotton, corn, soybeans, or sweet potatoes, these crops too have been observed to show symptoms of the same disorder.

SYMPTOMS.—Sand drown is usually first evident as the crop approaches maturity, following topping, when the leaves assume a characteristic yellow color. The yellowing begins at the tips and margins of the lowermost leaves, and the veins, as well as the tissue immediately adjoining, tend to remain normally green. It advances toward the base and central zone of these leaves until they are entirely blanched. The diseased condition grad-

ually spreads upward involving all leaves in turn from below upward. Affected leaves are dull and lusterless and become either very pale yellow in color or almost pure white, depending upon the acuteness of the deficiency. This yellow color may readily be distinguished from the clear, lemon color of healthy leaves which are partly cured. Cured diseased leaves have a dull, faded appearance, and the tips break away in handling during grading.

The Disease.—For the sake of exactness sand drown might better be called "magnesia hunger." The disease was recognized, however, for several years before its relation to a deficiency of magnesium was established, and meanwhile both growers and technical workers had come to accept the designation "sand drown."

History, Range, and Importance.—The disease first came under observation at the Tobacco Experiment Station, Oxford, N. C., in 1912, and has appeared there every year subsequently. It has been observed further to be present practically throughout the area with sandy types of soils in North and South Carolina. Garner, McMurtrey, Bacon, and Moss (1921) noted its occurrence also on the more sandy types of soils in the Connecticut Valley, especially in excessively wet seasons. It appears probable, too, that it exists in other tobacco-growing sections, in some cases, perhaps, in mild form and therefore not distinctly recognizable. In fields which are cropped year after year with tobacco, as is so common a practice in the flue-cured belt, sand drown is of increasing importance.

Garner and his associates (1923) have pointed out that sand drown lowers the market value of tobacco in a twofold manner: (1) by modifying adversely the presence of yellow and green pigments which are necessary in the development of proper color in the cured leaf, and (2) by interfering with the construction of cell walls and stored foods. As has been pointed out, cured sand drown leaves are brittle instead of elastic and leathery. Further, it has been determined that normal leaves are 45

FIG. 8. Rosetted plants with multiple buds and with leaves that are hooked downward at the tips, the result of withholding calcium from the nutrient solution in which the plant is growing.

FIG. 9. Sand drown or magnesia hunger in severe form on flue-cured tobacco. The yellowing and whitening begins on the lower leaves and spreads toward the top of the plant. The veins remain green. (From U. S. Dept. Agr.)

FIG. 10. Sand drown. A single leaf exhibiting the usual blanching of the tips and of the intervein tissues.

FIG. 11. Tobacco seedlings, the one in the middle is normal and those on either side, of the same age, are stunted and pale green from excess of boron.

per cent thicker and 60 per cent heavier per unit area than diseased leaves. Both quality and quantity are therefore adversely affected.

Etiological Relations.—As with the determination of the cause of nutritional diseases of animals, there are complications which render it difficult to determine the cause of deficiency diseases of plants. Although sand drown is known to be due to a deficiency of the element magnesium, it was first noted to be most marked in plots which received sulphate of potash in combination with other fertilizer materials. In plots which were otherwise comparable, except that muriate of potash replaced the sulphate, there was a striking decrease in severity of sand drown and an increase in size attained by the plants. Several series of tests were therefore made in which chlorides of potassium, magnesium, and sodium were contrasted with sulphates of these same elements, and at the same time with their carbonates, using chemically pure salts. In brief, these showed no control of sand drown except in the plots which received either sulphate of magnesium or chloride of magnesium. This observation gave the clue to the final solution of the problem which was accomplished by pot tests. Twelve-quart galvanized iron buckets were filled with either sandy loam from Prince George County, Maryland, or from Oxford, N. C. The plants in some of these pots were watered with a nutrient solution consisting of sodium nitrate, monocalcium phosphate, potassium sulphate, and magnesium sulphate. In some, potassium chloride was substituted for potassium sulphate; in others, ammonium nitrate was substituted for sodium nitrate; in others, magnesium sulphate was omitted from the nutrient, and it was in these last pots that sand drown appeared. When sand drown had not progressed too far on some of these plants, and magnesium sulphate was added to the nutrient, they made a rapid recovery. This showed that it was neither the sulphate nor the chloride but the lack of magnesium which was the cause of sand drown. Analyses of leaves and stems for magnesium content (Garner *et al.*, 1930) show

that there is more magnesium in the stalk than in the leaves and also that the content of magnesium in the leaves differs with position of the leaf on the stalk. When the plant as a whole has less than approximately 0.2 per cent of magnesium, deficiency symptoms are apparent.

Control.—Subsequent field trials at the Tobacco Experiment Station, Oxford, N. C., have shown that dolomitic or magnesian limestone will effectively prevent sand drown on fields whose soils are deficient in magnesium. The evidence from these tests, moreover, shows that from 20 to 50 pounds of actual magnesium per acre will suffice. It is advisable, however, to apply 1000 to 2000 pounds of dolomitic limestone per acre, dependent upon the character of the soil and the acuteness of the deficiency. This excess will remain for the subsequent crops and be efficacious over a period of three to five years. As an extreme case, it may be pointed out that in one plot to which a ton of magnesian limestone per acre was applied, sand drown was present in scarcely detectable amount in tobacco planted ten years afterward, whereas sand drown was quite pronounced on an adjoining plot to which no magnesian limestone had ever been applied. Judgment must be exercised in the matter of frequency of application and in the amount of magnesium which is added to soils, for the reason that black root rot, caused by *Thielavia basicola*, is favored by alkaline or only slightly acid soils, and has been found to be seriously destructive in fields in which it was never observed prior to the application of lime. In addition to these plot trials, there is abundant evidence, in increasing volume, from growers that the application of dolomitic limestone will effectively prevent this disease.

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Boron

General Considerations.—The element boron has for a long time been known to be present in minute amounts in a wide variety of soils, and also to occur in the ash of normal plants in quantity sufficient to permit of its chemical determination. More recently, it has been found that boron is absolutely essential to the normal growth of some kinds of plants, especially corn, broadbeans, and tobacco.

Curiously, while certain minimum concentrations of this element are indispensable to normal development, a very definite toxicity is apparent if the concentration is increased beyond a certain point. A diseased condition may therefore develop either from a deficiency of boron or from an excess.

The studies that have thus far dealt with nutritional disorders caused by boron include its influence on beans, peas, potatoes, corn, cotton, and tobacco. These crops vary in their sensitivity, none apparently responding more readily than tobacco. A deficiency of boron produces a diseased condition that may appropriately be called "top disease," a designation given by Kuijper (1930).

Symptoms.—The symptom-complex, as shown by McMurtrey (1929), is first apparent in the terminal growing point or bud. The leaf bases of the youngest leaves are lighter green in color than the tips. The failure of these tissues at the bases of the leaves to make normal growth results in the formation of leaves that are drawn and distorted. The affected tissues may become dry, or in extreme cases the entire bud becomes dry. The lateral buds, that normally remain dormant or appear only after the top has been removed, now unfold, or may themselves be similarly affected. Those leaves which have unfolded before the disturbance is apparent are stiff, brittle, and slightly yellow in color, and their vascular tissue is blackened. In a later report McMurtrey (1933) showed that plants lacking boron are dwarfed and that they fail to set seed pods because the flowers are shed.

In sand cultures deficient in boron, van Schreven (1933) found that there is a poor development of the root system, death of the growing point and axillary buds, thickening, yellowing and wrinkling of the leaves, with downward bending of the main and lateral veins, stunting of the entire plant, and discoloration of the tissues. In his anatomical studies he found that individual cells or groups of cells of the ground parenchyma, collenchyma, pericycle, phloem, and xylem become discolored and may eventually disintegrate. This accounts for the death of the buds. The enlargement of the leaf cells accounts for the increased thickness of the leaves. The extensive radial division of the phloem produces compression and distortion and results in curvature of the veins. The smaller size of the chloroplasts accounts for the yellowing. Starch translocation is impeded, and thus growth is impaired.

When an excess of boron is applied to the soil just prior to transplanting, the tobacco seedlings (Plummer and Wolf, 1920) may die soon after being set. Seedlings used to replace them will die, even if the soil is sufficiently moist to be favorable for growth. Such plants as do survive either fail to "grow off" or remain stunted. Those which start to grow possess leaves which are pale green, the yellowing being most pronounced in the parts most distant from the principal veins and along the leaf margins. These areas, in the severest cases, become dry, the leaf margins and tip turn downward, and at length the leaves become "rimbound."

The root systems of severely stunted plants consist of short fibrous roots all of which are clustered near the lower part of the main root; those plants which appear to be normal have, in addition, a cluster of roots near the ground level. This growth of secondary roots from the surface of the buried portion of the stem simulates the appearance presented by plants affected with black root rot.

The stand of plants in fields whose soil contains an excess of boron is very broken, and there is no uniformity in size as the plants approach maturity. The leaves

"fire" instead of ripening properly, resulting in a product inferior in quality.

History of the Disease.—Cases of boron deficiency in field practice are of limited occurrence. Apparently nearly all soils devoted to tobacco culture contain boron sufficient to provide against the occurrence of a boron deficiency disease. Kuijper (1930), however, reported a "top disease" of tobacco in Sumatra which he was able to overcome by the application of small amounts of a solution containing 3 mg. of boric acid per liter of water. Swanback (1927) was able to reëstablish the growing point in tobacco suffering from a deficiency of boron by the addition to the nutrient solutions of 4 parts per million of boron. The writer has observed what he regards as this disorder in limited proportions near Winston-Salem, N. C. If boron does not occur normally in the soil, since it is present in fertilizer materials, it is added along with the commercial fertilizer.

Recognition of a definite boron toxicity as a field problem dates with the use of borax (sodium tetra-borate) to kill the larvae of the house fly in manure. When such treated manure was used in liberal amounts under crops, it was found to be injurious.

The observation of injury to crops from the use of commercial fertilizer containing borax was first made in Indiana in 1917, where it caused serious injury to corn. During 1919 Plummer and Wolf (1920) noted its effect on tobacco, cotton, and corn in North Carolina, and several other investigators in the same year studied its effects on potatoes, beans, citrus, cotton, and other crops.

Boron was introduced in these commercial fertilizers as borax in the potassium salts. The sources of potash from Europe had been cut off because of the World War, so that domestic sources had to be employed. The inadvertent use of potassium salts containing borax from Searles Lake, California, resulted in damage to crops.

Factors Involved in Toxicity.—The experimental work done by various investigators under field conditions and in the greenhouse with pot cultures and nutrient solu-

tions, leaves no doubt about injury from boron. In the case of tobacco, normal growth occurs in concentrations of 0.4-0.5 parts per million of boron. Less than this proportion results in a deficiency; more, in a toxicity. The degree of injury depends upon several factors including the crop concerned, the amount of boron applied, the manner of application, the character of the soil, and the actual concentration of boron as modified by rainfall, leaching, adsorption by the soil, etc.

The toxic limit for tobacco is 1 pound of borax per acre; for wheat, 2 to 3 pounds; for beans, 3 pounds; and for corn, cotton, or potatoes, 5 pounds. These limits are for sandy soils, and larger amounts of borax are necessary to produce as marked injury in clayey soils.

"The presence of iron and aluminum in clay soils and the ability of borax to form insoluble compounds with these elements, especially under certain conditions, may in part account for the differences which appear in these two soil types. It is believed, however, the phenomenon of colloidal adsorption is an important factor in accounting for the tolerance of plants to larger amounts of borax in clay soils" (Plummer and Wolf, 1920).

In fertilizer practice the materials are applied in the drill row rather than broadcasted, and at the time of planting rather than at some time previously. These facts, as would be anticipated, exert an influence on the severity of the injury from the use of any given boron-containing fertilizer material.

All observations relating to the influence of soil moisture on injury due to boron are in accord. When plants were grown so that soil moisture could be controlled, a definite relationship was found to exist between the degree of injury and the per cent of soil moisture. Under field conditions, a heavy rain on sandy soils, by leaching action, resulted in little or no injury even with rather heavy applications of borax. The recovery of plants, as the season advanced, might also be interpreted as the result of a leaching out of the borax. The same activity, moreover, explains the field tests in which the toxic effect did not

persist from one season to the next, following the heavy application of borax in the first year.

Several theories have been advanced to explain the toxic action of boron: (1) that it acts as an anti-enzyme, preventing the formation of enzymes which render the reserve material in the seed available during germination and early growth of the seedling; (2) that it enters into chemical combination with sugars and other carbohydrates and in so doing interferes with the translocation of carbohydrates; and (3) that it induces chlorosis by combining with iron and thus interferes with chlorophyll formation. Even though this problem is not solved, all investigations are in accord in showing that boron exerts a profound influence on the mechanism of chlorophyll formation and on the essential nutritive processes in tobacco and other plants.

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CHLORINE

GENERAL CONSIDERATIONS.—Apparently it is not yet known whether chlorine is an essential element in plant growth. Many investigations have been concerned with whether chlorides are beneficial or harmful to a particular crop. The results of these investigations, as reviewed by Tottingham (1919) and by Lomanitz (1924),

show that the effect of chlorides depends partly upon such factors as the quantity of chlorine applied; the kind of crop; the type, reaction, and moisture relations of the soil; and the meteorological conditions that prevail throughout the growing season.

So far as the relation of chlorine to tobacco is concerned, this element is known to exert a profound effect upon the plant while growing in the seed bed and in the field, upon its resistance to drought, upon the quantity of the cured leaf, and upon the character of the manufactured product, especially of smoking tobacco. As the result of a series of comparative tests with sulphate of potassium and chloride of potassium on Durham sandy loam, Garner and his associates (1930) found an average increase of 10 per cent in yield on plots to which 20 to 30 pounds of chlorine per acre were applied. They assign two reasons for this stimulatory effect of chlorides: (1) the increased solubility of magnesium in the presence of chlorine, with the resultant absorption of sufficient magnesium to enable the tobacco plant to meet its nutritional requirements; and (2) the increased drought resistance, since the presence of chlorides within the tobacco plant enables it to better maintain its water content.

Effect of Excess of Chlorides.—Adverse nutritional effects are manifest when chlorine in excess of 100 pounds per acre is applied in the fertilizer, or in some cases when the quantity used is as low as 40 to 60 pounds per acre. The intensity of these effects from any given quantity of chlorine will manifestly vary with conditions. Most pronounced injury is likely to develop on sandy soils, low in humus content. Again, the amount and distribution of the rainfall modify the quantity of chlorine absorbed by the tobacco plant within any given period of time and consequently affect its growth.

Plants supplied with an excess of chlorine are dwarfed. This is especially pronounced on seedlings in the seed bed or on plants set in newly cleared land. The extraordinary thickness and brittleness of the leaves of such plants are strikingly characteristic, as is also the fact

that the margins of the leaves curl upward. Wilson (1933) has shown that the leaves of plants to which excessive amounts of chlorides have been applied are approximately two and one-half times as thick as normal leaves. The leaves of seedlings or the lower leaves of plants in the field may be so friable as to fall readily to pieces when handled. The leaf surface has a glossy, exceptionally smooth appearance, which is quite distinctive.

It is well known among growers that maturity is delayed by two weeks or more in crops that have received an excess of chlorine. When such a crop is flue-cured, the leaves are splotched with green, yellow, and brown, and are lacking in elasticity and toughness, and consequently have a lowered market value.

Garner and his associates (1920) have shown that the leaves of plants supplied with an excess of chlorine have a higher starch content, a higher water content, and a lower organic acid content than normal leaves; Wilson (1933) found that the osmotic concentration of their sap is less than normal ones, and that they have less bound water.

FIG. 12. Rolling of the leaves characteristic of chlorine injury.

FIG. 13. Tobacco leaves that are thickened and the margins rolled upward, resulting from excessive application of chlorides.

Apparently these effects on the growing tobacco plant are not due to a toxic action of chlorine but to an interference with the carbohydrate metabolism in which the water relations are involved, as pointed out by Garner and his associates (1930). When for any reason there is a loss of water in the cells of the green tobacco leaf, it has a decided effect in stimulating amylolytic processes involved in the conversion of starch to reducing sugars, as they were able to show. If, on the other hand, there was an increase in the water content of the cells, starch was caused to re-form. Starch is therefore digested in cells which are not turgid, and is re-formed when the cells have again absorbed sufficient water to become turgid. Since chlorides tend to maintain a high state of turgidity within the cells of tobacco leaves, we would expect the cells to be gorged with starch in the presence of excessive amounts of chlorides. Wilson (1933) has shown that certain salts of bromine and fluorine cause the foliage of tobacco to be sleek, greatly thickened, and brittle, and the margins of the leaves to be curved upward, resembling very closely the symptoms produced by chlorine. This might be anticipated because of the chemical relationship of chlorine, bromine, and fluorine.

Prevention.—Cured tobacco leaves must have an appropriate moisture content, desirable color, must be more or less elastic, and of good keeping quality. In addition, they must burn freely, in the case of cigar, cigarette, and pipe-smoking types. These qualities are either favorably or adversely affected by the quantity of chlorine present. The quantity of chlorine present is largely determined by the amount of chlorine applied in the fertilizer. The quantity of chlorine required for optimum yields and quality can only be known with exactness as the result of extensive trials in the field. Such experiments have not been performed in most tobacco-growing areas. For the southeastern United States, experiments and observations indicate that the chlorine content of fertilizers must not exceed two per cent for best results.

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Iron

General Considerations.—The necessity of iron for chlorophyll formation has long been known. When it is absent or supplied in inadequate amounts, plants lack their normal green color. In the case of tobacco grown under field conditions, a disorder due to a lack of iron has apparently not been noted.

Iron Deficiency.—Studies in controlled cultures, on the effect of a deficiency of iron on tobacco, have been made by Pinevich (1927) and by McMurtrey (1933). It produces a typical chlorosis, which is first observed in the bud leaves. The tissues between the veins are light green to almost white in color, and the veins tend to remain green. When seedlings are transferred to nutrient solution cultures from which iron is withheld, the lower leaves remain quite normally green; but the leaves of the bud, both intercostal tissue and veins, may eventually become perfectly white. No lesions, however, develop. Continued retention of such plants in cultures deficient in iron prevents stalk development and the formation of flowers. When plants that have developed pronounced symptoms of iron hunger are transferred to a solution to which iron has been added, they recover their normal green color in all subsequent growth.

Pinevitch (1927) noted that recovery of tobacco from iron hunger in water cultures is conditioned by the reaction of the culture solutions. When the culture solutions were rich in carbonates, recovery occurred with the employment of a variety of iron salts. Carbonates of cal-

cium rendered the solutions neutral or slightly alkaline. He explained the recovery of normal color in tobacco in slightly alkaline solutions containing iron salts by the delayed hydrolysis of the iron salts. This is supported by the observation that if solutions of iron salts in distilled water, made alkaline with NaOH to a pH = 9.6, are examined at intervals, iron may be discovered in the filtrate, and the solution is continuously becoming less alkaline. He also noted that plants show symptoms of iron deficiency when maintained in neutral or alkaline Knop's solution. A scale of chlorosis, ranging between normal plants on the one hand and severely disordered ones on the other, was obtained by varying the pH concentration.

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ARSENIC

Compounds containing arsenic have long been known to be injurious to plants. It is also well known that arsenicals are widely employed for sprays and dusts for the control of insects that feed by chewing. Some species of plants are considerably more tolerant of arsenicals, whether applied to the foliage and fruits or to the soil around the plants, than are other species. Tobacco is among those species which are not easily injured by arsenicals applied to the foliage.

Tisdale and Kelley (1927) noted a diseased condition of the stems of young tobacco plants, in the Florida-Georgia tobacco area, to which a poison mixture, consisting of maize meal and arsenate of lead had been applied to control bud worms, *Heliothis virescens*. It occurred after a light rain following a dry period. The lower leaves became mottled with yellow, accompanied by a stunting of the entire plant. Such plants were found to have dark brown to black stem lesions just at the surface

of the soil. These lesions, in some cases, girdled the stems, and the affected plants eventually died. When the lesions were small, they became sunken and dry, and the plants continued to make an impaired growth.

Examination of affected plants in the field revealed that lumps of the poison mixture were in contact with the injured parts and that these lumps were overrun with fungi. Isolations yielded *Aspergillus oryzae* (Ehrb.) Cohn, *A. niger* V. Tiegh., *Mucor* sp., and *Penicillium* sp. When a block of medium containing either of the two species of *Aspergillus* was applied to the stems of tobacco seedlings, injury resulted. Fragments of these fungi, however, when inserted into stems, were without harmful effect. They conclude that the injury is not directly due to the fungi, but is due perhaps to substances produced by the fungi while growing in the meal. Some species of *Aspergillus* are known to excrete toxic products in culture. The results of their experiments did not exclude possible injury from the arsenical.

As a preventive measure when arsenicals must be employed in budworm control, they suggest that the poison mixture be applied sparingly and that the soil be cultivated to prevent accumulation of the mixture in direct contact with the stems.

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Other Mineral Elements

Sulphur.—The sulphur requirements of the tobacco plant are provided for, in field practice, through sulphates added with the mineral fertilizer materials, and with products liberated by the decomposition of organic sources of nitrogen. Consequently sulphur deficiencies in tobacco culture have not been found to occur. Yields of certain other crops in some areas have been very markedly increased by applications of sulphur. When, however, tobacco plants are grown in cultures contain-

ing no added sulphur (McMurtrey, 1933), the younger leaves are paler green than normal. Characteristically the veins are light green in color while the intercostal tissues are less affected, in striking contrast with plants deficient in manganese in which the color of the veins is little changed but the intervein tissue is blanched, or with iron hunger in which all the leaf tissues may become perfectly white. If sulphur starvation is begun early in the development of the tobacco plant, it tends to retain its rosette form and fails to flower; if it is begun later, the effects on flowering and setting of seed are less marked. A period of two to three weeks, after transfer to conditions of sulphur deficiency, is required before symptoms are apparent. Recovery from sulphur starvation occurs almost immediately in plants that have been maintained for four to six weeks in solutions deficient in sulphur.

Manganese.—Manganese is one of those elements which is present in minute amounts in soils and in the ash of plants grown in manganiferous soils, a fact established by Scheele in 1774, in his *Opuscula Chemica et Physica*. Bertrand, in 1894, was first led to conclude that it is necessary for the growth of plants when he discovered that the sap of the lac tree owes its peculiar enzymic properties to the occurrence of manganese in this sap. A number of present-day botanists regard the element manganese as "stimulatory" to plants rather than necessary, because of its presence in them in minute traces. Kelley (1914) believes that manganese is a necessary element in plant growth that has not been recognized as such because it occurs as a contaminant in compounds of iron, phosphorus, and calcium. He ascribes to manganese two functions: (1) it acts as a catalyst, increasing oxidations both in the soil and within the plant cells; and (2) it tends to increase the absorption of calcium by the plant and decrease that of magnesium. An insight into the problem of the rôle of manganese in plants can be gained by consulting a series of reports by McHargue (1923).

Minute quantities of manganese salts applied to tobacco plants in sand cultures cause the formation of numerous lesions, smaller than a pinhead. The necrotic tissues become dark brown to blackish. This condition in the writer's opinion appears to be a toxicity, due to an excess of manganese. Jacobson and Swanback (1929) briefly described manganese toxicity to tobacco in water cultures containing 1 part per million of manganese, and in sand cultures containing 80 parts per million. They found a correlation between manganese content of plants and the reaction of the soil, the higher the acidity the greater the manganese content.

There are also very characteristic effects on tobacco from a deficiency of manganese (McMurtrey, 1933) which manifest themselves in a chlorosis of the younger leaves. The tissues between the veins are pale green, while the vein and veinlets retain their normal color. This contrasts sharply with the symptoms of a deficiency of

FIG. 14. Tobacco plants grown for six weeks in deficient culture solutions: 1, lacking nitrogen; 2, lacking phosphorus; 3, lacking potassium; 4, lacking calcium; 5, lacking magnesium; 7, lacking boron; 8, lacking sulphur; 9, lacking manganese; 10; lacking iron; and 6, containing all of the above-named elements. (From McMurtrey.)

sulphur in which the veins are blanched and the intervein tissue is little changed. The venation thus forms a checkered pattern on a whitish background. In later stages small dead areas appear that dry to a light brown or whitish color.

McMurtrey (1933) also points out that the development of symptoms of manganese deficiency requires four to five weeks after plants are transferred to solutions to which no manganese salts have been added. In the writer's opinion the reason for this delay is the fact that many "chemically pure" chemicals contain traces of manganese, and that manganese appears to be available to plants only in acid substrata.

Cyanamid Injury.—Calcium cyanamid is one of the synthetic compounds employed in mixed fertilizers as one of the sources of nitrogen. Field trials have shown that the proportion of calcium cyanamid that can be used with safety under tobacco should not exceed 0.5 per cent. This means that one-eighth to one-tenth of the total nitrogen may be derived from calcium cyanimid. If cyanamid in excess of this proportion is applied to tobacco on light sandy soils, yellowish, indefinitely limited specks appear on the leaves, especially those near the top of the plant. These specks tend to occur near the leaf tips and margins, and the tissues may break down as maturity approaches. This condition appears to be a toxicity. The symptoms are very readily recognizable to one who has once seen them.

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IV

Diseases Due to Unfavorable Water Relations

Introduction

The factors that control the availability of water to plants are of enormous importance in governing the natural distribution of seed plants upon the earth's surface. Those plants which grow in the water or in swampy situations are ecologically classified as hydrophytes, those which occur in deserts are xerophytes, and those which are intermediate between these extremes in their water relationships are mesophytes. Tobacco and, in fact, most crop plants are in this last category.

Before entering into a discussion of the effects of a lack of sufficient water, or of an excess of water on the tobacco plant, one may well recall and keep in mind the rôle of water in plants in general. The brief axiomatic résumé, that follows, of the uses of water by plants is pertinent to a correct understanding of water relationships.

Uses of Water

(1) Plants are largely constituted of water, since it comprises from 80 to 98 per cent of their weight. (2) Water is a universal solvent, so that watery solutions of mineral elements enter plants and pass throughout the plant body. (3) Water is one of the crude materials, which in the process of photosynthesis, enters into the formation of sugars and starches. The carbon, hydrogen, and oxygen from these carbohydrates may in turn be synthesized into proteins and fats. (4) The digestion and transport of food within the plant require the presence of water. (5) Water is necessary for turgidity

of cells, a condition which is essential for growth. (6) Water makes possible the action of enzymes and lubricates chemical reactions within cells. (7) Water is absorbed largely by the roots, and is lost by transpiration from aerial parts. The rate of transpiration is an index of the rate of growth and the accumulation of organic compounds within the plants so that water may be said to regulate growth.

Usual Effects of Water on Tobacco

The quality of cured tobacco of any particular type is dependent largely upon the amount and distribution of rainfall to the growing crop. Other potent factors include physical and chemical properties of the soil, kind and amount of manurial materials applied to the soil, temperature, and sunshine. Frequent light rains favor the normal development of the tobacco plant. Shortage of water throughout the season results in retardation of growth and dwarfing. It is very difficult to age flue-cured tobacco grown under these conditions, and its nicotine content is abnormally high. An excessive supply of water stimulates excessive growth, and in the case of flue-cured tobacco, the leaves are thin, papery, and may appropriately be characterized as "washed out." They may be bright colored, but are almost wholly lacking in aroma, elasticity, and body.

All growers are familiar with cases of sudden acute deficiencies of water to the growing crop, which manifest themselves by wilting. These occur during periods of high temperature and intense illumination, or when the roots have been disturbed in cultivation. In this case the loss of water from the leaf surface exceeds that absorbed by the roots, in consequence of which there is a decrease in turgor of the cells, and the leaves then become flaccid. Such plants recover their turgor during the night, or when absorption and transpiration have been equalized.

During a heavy rainfall, especially when the rain is accompanied by rather strong winds, water is driven into

the leaves through the stomates, and irregular, translucent splotches are formed. These are most noticeable, especially on the lower leaf surface, if examination is made shortly after the storm has ceased. These splotches may disappear, on drying, with no permanent ill effects. If, however, the bacteria that cause wildfire or angular leaf spot are driven into the leaves during rain storms, large lesions quickly result. Sometimes scalded areas appear on such leaves, particularly when intense sunshine follows immediately after the rain. Damage, in this case, may be due in part to the fact that the drops of water on the leaves may focus the sun's rays and thus act like a lens or burning-glass.

Deficiency of Water

Drought Spot, Appearance.—When the moisture supply of tobacco is deficient for protracted periods, a condition which may be referred to as "drought spot" develops. This condition is characterized by the death of the tissues between the veins. Large circular to irregularly lobed spots are formed, which vary in size from 10 to 20 mm. These spots are brown, with a definite darker border, and are surrounded by a zone that is yellow and that pales out into the normal green of the leaf. When the spots are large enough or sufficiently abundent, they coalesce to form large irregular patches. The margins of leaves affected with drought spot tend to curl downward. In extreme cases the leaf margins, beginning in the portions most distant from the veins, become dry. This manifestation is known by growers as "rim fire."

Contributory Factors.—Garner and his associates (1930) have pointed out that there is a striking protective action against drought spot afforded by chlorine applied as chlorides. This was apparent in 1925 at the Tobacco Experiment Station, Oxford, N. C., in the potash plots. To these plots the equivalent of 40, 80, or 160 pounds of K_2O per acre was applied. Drought spot was very severe on those plots which received 40 to 80 pounds of potassium sulphate (sulphate of potash), but was

slight in those which were given 160 pounds. Those to which potassium chloride (muriate of potash) was applied were not materially injured, most damage being apparent on the plot to which 40 pounds were applied. It appears that both potash and chlorine exercise a protective rôle against drought, but Garner and his fellow workers (1930) were led to conclude that chlorine is decidedly more effective, because "potash as such failed to protect the leaves from burning when present in the tissues to the extent of 3.5 per cent of the dry weight and accompanied by a chlorine content of less than 1 per cent. When the chlorine content was increased to 3 per cent or more, evidence of injury was practically absent. Again, with a chlorine content of about 0.8 per cent but with an increase in potash content to 5 per cent there was effective protection." The writer has noted numerous

FIG. 15. Acute deficiency of water to young plants, resulting in large, irregular, dead areas on the leaves.

FIG. 16. Symptoms of drought spot on the mature crop. Brown spots surrounded by yellowed tissues are most abundant at the leaf tips.

instances in the crops of growers in which muriate of potash was exercising a beneficial effect during drought. Fromme (1928) has pointed out that chlorine applied as salt (NaCl) aids in preventing injury to tobacco when periods of hot, dry weather prevail during the growing season.

That chlorine enables the tobacco plant to resist desiccation was borne out by the determinations of the water content of leaves of Maryland tobacco by Garner and his associates (1930). Their data on this point are as follows:

TABLE 3

EFFECTS OF CHLORINE IN FERTILIZER ON THE WATER CONTENT OF TOBACCO LEAVES

Plat No.	Quantity of chlorine in mixed fertilizer	Potash in fertilizer			Water content of leaves and date of analysis					
		Quantity per acre	Form		July 20	Aug. 3	Aug. 10	Aug. 26	Sept. 2	Sept. 9
			Sulphate	Chloride						
	lbs.	lbs.	lbs.	lbs.	per cent	per cent	per cent	per cent	per cent	per cent
1	0	40	40	0	81.68	85.66	82.20	84.23	83.77	83.49
2	202	40	0	40	85.35	88.88	84.38	86.73	85.99	84.40
3	0	80	80	0	82.76	85.76	79.49	82.39	77.32	78.78
4	130	80	0	80	86.14	89.37	83.04	85.16	81.16	80.19

These data consistently show that the plants to which chlorides were applied had the higher water content. Such differences in water content are manifestly correlated with other significant differences in cell metabolism and in chemical composition. It is of interest to note, too, that there is a downward trend in the water content of leaves as they approach the stage for harvesting.

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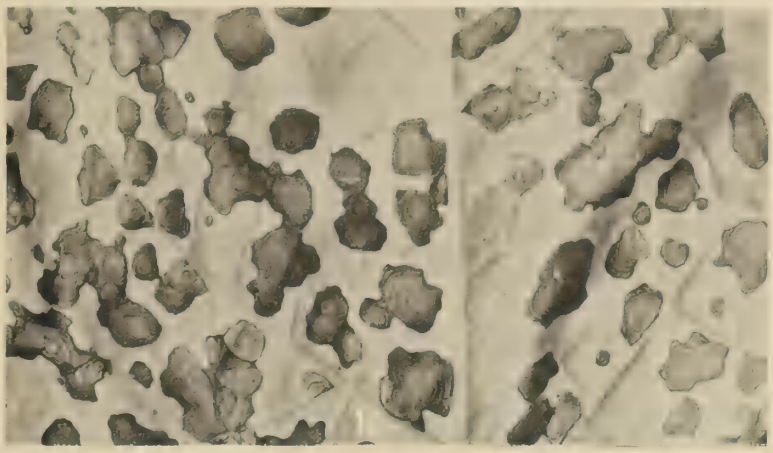
Excess of Water

Wet Weather Spot.—Just as there is a relationship between a marked deficiency of rainfall, chlorine content of the leaves, and "drought spot," so there appears to be one between an excess of rainfall, chlorine content of the leaves, and a condition which may be designated as "wet weather spot." In its gross symptoms wet weather spot is quite similar to drought spot, but it is of course the sequel to prolonged wet weather or to a series of heavy rains. The physiology of breakdown of cells and of the resultant formation of large, brown, dead areas, following wet weather, remains to be investigated, and is a very important matter, especially in areas devoted to the culture of flue-cured tobacco.

Blackfire.—Growers of White Burley and dark-tobacco in Kentucky and Tennessee have for a long time employed the name "blackfire" to a disease which is very destructive there in wet seasons or in poorly drained portions of the field. It is very probably, in the writer's opinion, that blackfire of Burley tobacco is identical with "wet weather spot" of flue-cured tobacco. Valleau (1932) described it as characterized by spots that are at first water-soaked in appearance and that have concentric zonations as they enlarge, and the centers become dry. These lesions are generally found to be free from bacteria. Blackfire may start rather suddenly and spread rapidly after heavy rainfall or after the crop has been topped.

Valleau (1932) records that it is usually absent from well-fertilized fields whose soils are in a good state of tilth. Dark tobacco grown on fields liberally supplied with organic matter or with stable manure is not affected with blackfire.

Burley growers have come to believe that as long as the crop is growing uniformly there is little likelihood of the development of blackfire. If growth is disturbed by topping or by heavy rains, the disease may appear. They therefore have adopted the practice of not topping until time for harvest, or permitting one or two suckers to de-



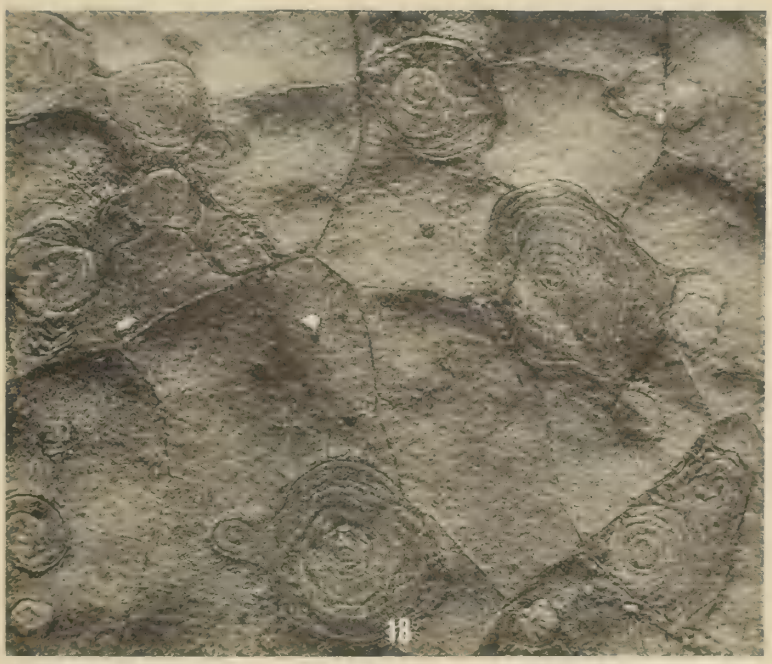


FIG. 17. Concentrically zonate lesions of blackfire on Burley tobacco leaves. (From Valleau.)

FIG. 18. Blackfire lesions on flue-cured tobacco. (From Wingard.)

velop in the top of the plant and allowing them to remain and grow until the crop is cut.

REFERENCE

VALLEAU W. D., Tobacco diseases in Kentucky. KY. AGR. EXP. STA. 328: 109-154. 1932.

FLOP.—Another little understood condition which is the sequel of heavy rains is known by growers as "flop." It occurs when fields are flooded, or when water remains standing between the rows for hours after the rain because of inadequate ditching or because of the impervious nature of the subsoil. The term "flop" is descriptive of events to the tobacco plant. The leaves of affected plants suddenly droop in an arching manner, being supported partly by the midrib. The laminar tissues are completely wilted and flaccid. Apparently flopped plants never completely recover. If they regain their turgidity after a few days, they fail to make any appreciable subsequent growth. Usually the leaves gradually dry up, and remain attached, and the plants have the appearance of having been killed by the bacterial wilt organism, *Bacterium solanacearum*.

No studies have been made of the proximate cause of death in the case of flop in tobacco. Apparently death is not due simply to drowning, that is, to a lack of oxygen. This opinion is based upon the fact that tobacco thrives in water cultures, especially if they are aerated by bubbling air through them or if algae are growing in sufficient quantity in the cultures to aerate them. It may be due to the excessive accumulation of carbon dioxide in the soil immediately surrounding the roots, and its absorption into the plant body. Carbon dioxide, derived from respiration within the roots and from decomposition of organic matter within the soil, should be absorbed by the water in the sodden soil, and its concentration should accumulate to the point of saturation. That death of tobacco may be due to toxicity from carbon dioxide is indicated by the fact that such is known to be the case with several other kinds of plants.

V

Disorders and Diseased Conditions of Tobacco That Are Little Known and Non-Infectious

Tobacco is subject to a number of disorders that are of peculiar interest because their causal agencies or factors are not understood, because their causes are largely conjectural or insufficiently investigated, or because they are of infrequent occurrence. Some of them are of considerable importance, especially the brown root rot disease and frenching, and all are of great interest scientifically. Further investigation may reveal that they should properly be placed under other captions, and they are admittedly not related to each other in the sense that their causes are similar. They are grouped together in this account mainly for the reason that they are not known to fit into any of the other groups.

Brown Root Rot

Our knowledge of brown root rot of tobacco is largely the result of investigations conducted in the Connecticut Valley subsequent to 1915. Undoubtedly it existed there prior to this date, but was confused with black root rot, caused by *Thielavia basicola*, from which it cannot readily be distinguished when both disorders are present simultaneously. The gross appearance of the above-ground parts of the plants affected with brown root rot and black root rot is quite alike, although the symptoms on diseased roots serve as a means of distinguishing the two disorders.

Suscepts.—Brown root rot has been observed on shade-grown Cuban and sun-grown Havana Seed and Connecticut Broadleaf tobacco in the Connecticut Val-

ley. Other varieties, especially those resistant to black root rot, have been tested in brown root rot "sick" soils with no evidence of resistance to brown root rot (Johnson, Slagg, and Murwin, 1926). Whether or not different species of *Nicotiana* vary in susceptibility to this disease has not been determined.

When tomatoes are planted in "sick" soils, they, too, exhibit root lesions like those on tobacco and appear to be equally as subject to attack. Such other solanaceous plants as potato, eggplant, and pepper, moreover, are affected but not so seriously as tomato. Further, such leguminous crops as cowpeas, soybeans, garden beans, hairy vetch, and the clovers are moderately susceptible when grown in brown root rot infested soil. Whether or not cereals are affected adversely on such soil has not been convincingly determined.

Symptoms.—Brown root rot does not ordinarily attack plants uniformly over large areas, but is limited to spots in the field, although sometimes all plants in a field except those in a few small patches are involved. The most striking above-ground symptom is the general dwarfing of plants, which may show a decided tendency to recover toward the close of the growing season. During periods of drought or excessive water loss, dwarfed plants may wilt, a symptom which is more pronounced on sun-grown than on shade-grown plants. Wilting is accompanied by a gradual premature yellowing and death of the lower leaves. Since stunting, wilting, and yellowing of the leaves are also characteristic of black root rot, a positive diagnosis can only be made after an examination of the root system. Severely affected plants are easily pulled up, and the few roots which remain are brown and decayed; the roots of less severely diseased plants, especially those near the surface of the soil, have distinct, well-developed, brown lesions, while the roots arising from the base of the stalk are poorly developed. There is thus a tendency, as in black root rot, for affected plants to form new roots near the surface of the soil following the destruction of the main root system. The lesions are

brown, however, instead of black, as in the black root rot, and microscopic examination of brown root rot lesions fails to reveal spores on the surface of the decaying roots.

The Disease; Historical.—Brown root rot of tobacco was first described in 1919 by Johnson, who then believed that a species of *Fusarium* might be the causal organism. The next year Clinton (1920) in Connecticut described a "red root rot" of tobacco, and Chapman (1920) in Massachusetts recognized the presence there of a disease distinct from black root rot. Both investigators were undoubtedly dealing with this brown root rot disease. It is evident that Garner, Lunn, and Brown (1925) were dealing with essentially the same problem in Maryland, since they found that there are deleterious residual effects of certain crops on succeeding crops of tobacco. A condition similar to brown root rot has been recorded by Johnson (1926) in Wisconsin and Kentucky, and in all likelihood this disease exists in other tobacco-growing areas. Apparently it is rather common in North Carolina, where it has been confused with the black root rot disease.

Cause.—The actual cause of brown root rot has not been definitely proved. Johnson and his associates (1926) found by planting on culture media tissues of diseased roots that a species of *Fusarium* could be obtained consistently in a large proportion of the plantings. Negative evidence of pathogenicity, however, resulted from their attempts to secure infection by placing this *Fusarium* on the roots of healthy plants, or by contaminating soil with it and then transplanting healthy seedlings into this infested soil. Since species of *Fusarium* are soil inhabitants, and many of them are known to be saprophytic, their presence in decaying roots or on healthy ones is to be expected. Moreover, Johnson and his associates (1926) isolated a species of *Rhizoctonia* and of *Actinomyces*, both of which genera contain pathogens, but neither produced infection when used in inoculation experiments.

Even though no specific organism has been proved to be the cause of brown root rot, the following evidence points toward the existence of a causal organism:

(1) Plants set in "sick" soil, even when mixed with "healthy" soil in proportions as low as 10 per cent, become affected with brown root rot.

(2) Plants develop brown root rot when set in healthy soil which has been contaminated by the addition to it of macerated diseased roots.

(3) Healthy plants are produced when "sick" soils are sterilized by steam or by formaldehyde and then healthy plants are transplanted into them.

As opposed to this evidence of a specific causal organism Johnson and his co-workers (1926) noted the following facts:

(1) When "sick" soils were spread out in a thin layer and were rather rapidly dried, even in the absence of direct sunlight, they completely lost, within one to six days, their ability to produce brown root rot. This is not known to be true of soil-borne fungous or bacterial pathogens, associated with any other plant disease.

(2) In rotation experiments when tobacco followed tobacco on "sick" soils, brown root rot tended to disappear. If, however, tobacco followed certain crops which are not subject to brown root rot, especially timothy, the disease tended to increase in severity. This is, of course, contrary to what would be expected if some organism were the cause. It would appear from this that the residues of preceding crops, on decomposing, give rise to substances toxic to tobacco. Doran (1928) submitted evidence in support of this explanation of the cause. He showed that infusions of timothy, dependent on the stage of decomposition, when added to soil in which tobacco was growing, produced lesions indistinguishable from brown root rot. Thomas (1930) showed that there is a relationship between the presence of cellulose materials, nitrates, and brown root rot. Soils which have an abundance of cellulose produce brown

FIG. 19. The plants in this field were stunted by brown root rot. The plant on which the seed head is bagged remained healthy, but the plants grown from its seed exhibited no evidence of resistance to brown root rot. (From James Johnson.)

FIG. 20. Frenched tobacco plant, rosette-like, with numerous narrow leaves. The succeeding crop in this field was normal in that portion to which sulphur was applied.

root rot, and those free from brown root rot have an abundance of nitrates early in the season. His evidence indicates that certain soil fungi which attack cellulose, may, if nitrates are absent or are present in insufficient amount, attack tobacco roots while these fungi are in search for nitrogen, and thus cause brown root rot. More recently Swanback and Jacobson (1933) have procured evidence that a form of brown root rot is due to the insufficient intake of calcium.

Control.—Observations indicate that there is little likelihood of sufficient varietal differences in susceptibility to lead one to hope ever to be able to employ the culture of resistant varieties as a means of control. Further, infested fields tend to recover without any special treatment.

The experimental work which has been conducted to determine the proximate cause of brown root rot has included field tests on the influence of crop rotation systems, fallowing, liming, and the application of commercial fertilizers, on the prevalence of the disease. None of these factors except the first one is of especial significance. An interpretation of the results shows that lime has a slightly detrimental effect on tobacco grown on infested soils, but with no correlation between soil reaction and incidence of the disease.

In the rotation tests, timothy, corn, and clover when grown in brown root rot infested fields were injurious to subsequent crops of tobacco. When any one of these three crops followed itself or the others on infested soils, no such injury was apparent. On other fields, not so infested, there was no injurious action on subsequent crops of tobacco. This must be interpreted to mean that on certain soils the cropping system bears a contributory rather than a direct causal relationship. When continuous culture of tobacco was employed, brown root rot decreased in severity. Such a system of culture with tobacco would in time lead to the increased prevalence of other disorders of this crop.

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Frenching or Polyphylly[1]

Frenching, which has appropriately been called polyphylly, occurs in all tobacco-growing regions of the world. Although it was the first disease of tobacco to be clearly described, its cause has not been satisfactorily determined. The investigations to determine the cause of this disease have, in many cases, been fruitless, and the results have not been published. The results of the studies by various investigators that have been published, together with those of the writer and his associates that have not appeared in printed form, are assembled herewith as a contribution to our knowledge of the problem of frenching of tobacco.

History of the Disease.—Frenching has been known presumably ever since the days when tobacco was first grown by the American colonists. The following account of it was prepared by John Clayton, at Wakefield in Yorkshire, Va., in 1688:

> French-men they call those plants whose leaves do not spread and grow large but rather spire upwards and grow tall. Those plants they do not tend, being not worthy their labor; were they so critical I believe they might have great guess what plants were likely to turn French-men, by observing whether the roots of the plants run downwards as those branches are aptest to spire upwards. For though I have not made positive proof

[1] The writer has freely drawn upon an unpublished manuscript prepared by Dr. G. A. Brecher, while he was a graduate student at Duke University, for materials for this report. Grateful acknowledgment is hereby made. The writer is also indebted to Dr. John Ehrlich and Mr. L. B. Wilson for notes taken from their studies.

thereof, I have something more than bare fancy for my conjecture; I have pulled up some of these French-men and compared them with the roots of some other plants and found them much longer than others; and 'tis observable, loose soil and sandy ground are more subject thereto than stiff land.

Frenching was first reported in Europe, in 1857, by van Swieten, who apparently included it under the disease named *roest* (*rust*, in Dutch). A free translation of his account states that growers ascribe *roest* to a poor growth of young plants produced by a subsoil so hard that the roots cannot penetrate it. Others believe that it appears when the weather conditions, especially cool nights, make the plants tender.

The report of the United States Department of Agriculture for 1874 (Dodge, 1875), contains the following reference to the disease:

A second cause (of abnormality) is too much wet weather after the plant starts to grow, causing it to French as we term it; the leaf thickens, grows very narrow, dagger-shaped, frequently not broader than a case knife and often as many as fifty leaves to the plant, all of them spread out on the ground. In this case the best plan is to pull the plant up, as it is worthless.

In 1880 Killebrew briefly summarized available information on frenching in a paragraph, whose context is quite like that in a subsequent revision (Killebrew and Myrick, 1928) which states:

Frenching (from the French word *friser*, to curl) attacks tobacco upon old clayey lands inclined to be wet, that have been much compacted by the trampling of stock, or through other means. Rainy weather is also a predisposing cause to the disease, and it sometimes manifests itself over a considerable area, but if tobacco is closely plowed and a vigorous pull is given to the plants so as to break the tap root, a large majority of them will recover if treated before the disease has gone too far. The first appearance of the disease is seen in the buds of the plants, which turn to a honey-yellow color. As the leaves expand they become thick and fleshy, growing in long, irregular, narrow strips with ragged outlines, the leaves often cupping downward.

When cut and cured such leaves are lifeless, with a dingy, dead color and are very light in weight. Frenched tobacco is worthless for any purpose except as a substitute.

In a number of accounts frenching and mosaic appear to have been confused, as witnessed by the publications of Iwanowski (1893, 1899, 1903) in Russia, Koning (1900) and Heintzel (1900) in Germany, Delacroix (1906) in France, Hunger (1905) in the Dutch East Indies, and Jenkins (1914) in Connecticut.

Preissecker (1909, 1915), from investigations in Dalmatia and Galicia, was among the first European workers to recognize that frenching and mosaic are distinct disorders. This distinction was maintained in Peters and Schwartz's (1912) compilation of the diseases and insect enemies of the tobacco plant. Jensen's (1913) investigations in the Dutch East Indies maintain that frenching has been known there since 1898, but he himself, even as late as 1921 (Jensen, 1921), was not clear on the distinction between frenching and mosaic, as shown by the following description:

Tjakar. Oorzaak oonbekend; waarschijnlijk dezelfde als bij mosaikziekte. Het Javaansche woord "Tjakar" beteekent: Kippenpoten, een zeer kenteekende naam voon de tabaksbedenen, die aan deze ziekte lijden. Zij worden smal, en op beide zijden van de middennerf is de smalle rand van de bladschijf omregelmatig gekorven, zoodat hetblad wel lijkt op enteen met omregelmatige huidplaatijes op beide zijden. In minder geprononceende gevennen is de bladschijf minder gereduceend, en alle mogelijke overgangen tot bet volkomen normale blad kunnen gevonden worden.[2]

Names of the Disease.—Two explanations have been made of the significance of the name *frenching*. One is that it is derived from the French word *friser*, to curl (Killebrew, 1880), because the leaf margins and surface of diseased leaves is undulating or wavy. The other is

[2] "*Tjakar*. Cause, unknown; evidently the same as mosaic. The Javanese word *Tjakar* signifies chicken feet, a very apt name for this disease. The laminar tissue on both sides of the midrib forms a narrow, irregular, wavy band. In less severe cases, the blade is less reduced and doubtless all intergrading conditions to normal leaves exist."

that "French-men," as used by the English Colonists and as modified to "frenching" by present-day growers, originated as an expression of derision in which comparison was made of the military bearing assumed by the undersized French soldiers and of diseased plants bearing slender, spindling, erect leaves. It should be recalled that in colonial days an intense antipathy existed between the French and English.

Van Swieten (1857) employed the name *roest* to the disease as it occurs in Netherlands. Konig (1900), in Germany, applied the name *schmalblatterigkeit* (narrow-leafed) and Polowtzov (Iwanowski, 1903), the name *schurgal* to the disease as it occurs in Bessarabia and Kachetia. In France, Delacroix (1906) termed the disease *polyphyllie* (many leafed), Preissecker (1909, 1915), in Austria, the German terms *schwertblatt* (sword leaf)

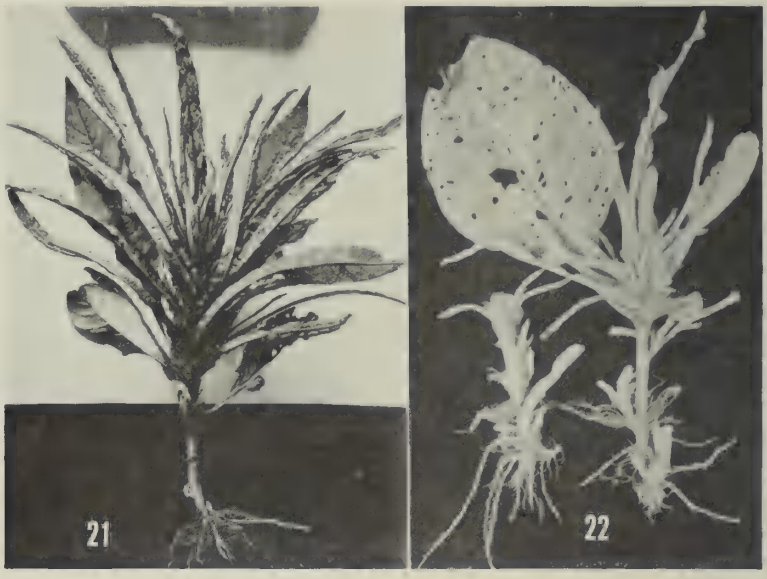

FIG. 21. Usual type of frenched plants, with thick, erect, narrow leaves. The white buds near the base of the stem arise beneath the surface of the soil.

FIG. 22. Severely frenched seedlings that had been transplanted about five weeks, and are now rosette-like. White buds have formed on the portions of the stems beneath the surface of the soil.

and *vielblatterigkeit* (many leafedness), as is accepted also by Peters and Schwartz (1912) and Kissling (1919). More recently Jensen (1921) and Jochems (1926) employ the Javanese word *tjakar* and the Dutch word *kippenpoten* (chicken feet). Clinton (1914) used such descriptive names as "gray top," "sword leaves," "string leaves," "stringy top," "shoe strings," and "phyllodination." The name applied in Cuba is *macho* (literally translated, male) since maleness is associated with infertility and hence worthlessness. Recently Schweitzer (1933) used the name *tjemara* because the shape of the leaves is like that of *Casuarina equisetifolia*, which is called *tjemara* in Java.

Suscepts.—All varieties of cultivated tobacco, *Nicotiana tabacum*, are susceptible to frenching. Little attention has been given to the matter of specific and varietal susceptibility in *Nicotiana*. Valleau and Johnson (1927) observed the disorder in F_1 hybrids of *Nicotiana rustica* X Burley and in *N. repanda*. Doctor Brecher observed frenching in the following species of *Nicotiana*: *N. clevelandii*, *N. chinensis*, *N. repanda*, *N. rustica* var. *humilis*, *N. suaveolens*, *N. sylvestris*, *N. tomentosa*, and *N. trigonophylla*. The following species developed no symptoms of frenching when grown under similar conditions: *N. bigelovii*, *N. cavanillesii*, *N. glutinosa*, *N. palmeri*, *N. plumbaginifolia*, and *N. rusbyi*. Such other solanaceous species as potato, pepper, tomato, and ground cherry (*Physalis wrightii*), when grown in soil in which tobacco became affected, remained free from frenching. Petunias and tomatoes became frenched, however, in frenching soil used by Karraker and Bortner (1934). Tobacco, when grafted onto Jimson weed (*Datura tatula*) stems, has been noted by the writer to be frenched. Non-solanaceous species, including citrus, corn, buckwheat, hemp, mustard, and sunflower, also showed no symptoms of frenching when grown in soils known to produce the disorder in tobacco.

Range.—Frenching has been reported in all tobacco-growing districts; hence its occurrence would not seem to

be related to soil types. It affects tobacco year after year when grown in the same areas, and the symptoms vary in severity. These areas may contain only a few affected plants or be so extensive as to involve practically all plants on areas comprising several acres. "Frenching soils" are usually low-lying, with poor surface drainage, and are underlain with very compact, impervious clay, called hard-pan, through which water passes with exceeding slowness. Occasionally a few normal plants have been noted in situations in which all neighboring plants were frenched. Investigation of soil conditions in these cases reveals that the healthy plants stand on sites in which large stumps have been removed, and in which the soil is deep, whereas the hard-pan is near the surface of the soil under surrounding frenched plants.

Symptoms.—Both frenching and mosaic are characterized by chlorosis and leaf deformities, both may occur on the same plant simultaneously, and hence have been confused in many earlier investigations. The former is non-infectious, however, and the latter is readily communicated from plant to plant.

Fig. 23. Portions of two adjacent rotation plots at the Tobacco Experiment Station, Oxford, N. C. Tobacco always becomes frenched on the one, the check plot, to which no commercial fertilizer is applied, when it is cropped with tobacco every fourth season.

Fig. 24. Potted frenched plant that was removed from the field a few days before being photographed. The three largest leaves are quite normal. The plant had been topped low because it was frenched. All of the leaves that developed subsequently were of the string-leaf type.

Frenching occurs on tobacco both in the seed bed and in the field. It has been noted on seedlings two to three weeks old. It may first become evident on the earliest leaves to unfold following transplanting or may appear at any subsequent stage in the development of the plant. Crops have been noted that remained free from the disorder until after topping, when all of the suckers became frenched.

In the mildest form of frenching, especially in the rapidly growing crop, there occurs a chlorosis of the bud leaves from which the recovery may be complete after a few days. Mild forms of the disease may properly be designated the "yellow-top" phase. In severest cases chlorosis is more marked, and the laminar portions of the

FIG. 25. Normal tobacco plants, all of whose leaves except the tips had been removed by priming, showing the extent of the root systems. (Compare with FIG. 26.)

FIG. 26. Two severely frenched plants that were grown about 10 feet distant from those shown in FIG. 25. The root systems of frenched plants are seen to be dwarfed. The larger plant bore over two hundred leaves.

leaf blade are inhibited from developing, so that the mature leaf consists of the midvein with only narrow strips of blade on either side. This form is designated the "string leaf" phase of frenching. In plants in this condition, the internodes remain short, apical dominance is lost, and an unusually large number of leaves appear, oftentimes two to three hundred on a single plant. Such plants have the appearance of a rosette or witches' broom, and therefore the severe form of frenching may properly be called "polyphylly." In intermediate cases, the leaves attain a width of 2 to 3 inches, and this is called the "sword leaf" phase. The tips and margins of unfolding narrow leaves are hooked downward. Such leaves are stiff, thick, and stand erect. The margins are ruffled, and their surface is undulating. Although the foliage is at first chlorotic, practically all appearance of chlorosis may disappear, even in the string leaf and sword leaf phases.

FIG. 27. Seedlings grown in frenching soil become affected early. The first leaf may exhibit symptoms of frenching. These seedlings are about five weeks old.

The complete symptom-complex of frenching includes all gradations between the conditions just described and variations between these types. One and the same plant, for example, may have normal leaves, yellow top leaves, sword leaves, and string leaves. Four to six lower leaves on some plants may be entirely normal, and the entire top may have the appearance of a witches' broom. The leaves on other plants may be progressively more severely affected from the oldest to the youngest. Branch buds do not normally develop on those portions of the stems of tobacco plants that are beneath the surface of the soil. When, however, plants are severely frenched, these buds develop; and if the plants are uprooted, they may be found as white outgrowths. As soon as they push above the surface of the soil they become green.

An appreciation of the extent to which frenching modifies the morphology of the tobacco leaves can be gained from a graph, Fig. 35, prepared from measurements of the length and width of approximately five hundred string leaves, and an equal number of sword leaves in comparison with the same number of normal ones. Each pyramid graphically represents either the leaf width together with the number of leaves having this width in a population of five hundred leaves, or the leaf length in the same population. The average length of normal leaves is seen to be approximately 55 cm., and the width, 30 cm. The corresponding measurements for sword leaves are 40 cm. and 10 cm., and for string leaves 27 cm. and 1.8 cm.

The effect of frenching upon the stems and leaves is no more pronounced than those upon the root system. These differences are contrasted in Figs. 25 and 26. Figure 25 shows two entire plants from which the lower leaves had been harvested, and Figure 26 shows two frenched plants equally as carefully removed from a site less than a rod distant. It is apparent that in frenching the inhibition of the root and top development are correlated.

Histological Effects.—Approximately a hundred leaves each of the yellow top, string leaf, and sword leaf

FIG. 28. Characteristic yellowing and cupping of the bud leaves of frenched plants. This plant had been transplanted into frenching soil about a month, and all of the new growth, subsequent to transplanting, became frenched.

FIG. 29. Young frenched and normal leaves of the same age. The edges and tip of the frenched leaf are turned downward, and the entire intervein portions are yellowed.

FIG. 30. A normal leaf and a sword leaf of the same age. The leaves were hung against a window pane to be photographed, consequently the pattern exhibited by the sword leaf is that seen by both transmitted and reflected light.

FIG. 31. String leaves of different ages and an adventitious bud whose leaves are all string-like.

phases of frenching were sectioned and stained in comparison with normal leaves of the same age in studies involving the anatomical changes in this disorder. These show first of all that diseased leaves are thickened, and in the mature condition range from two to three times as thick as normal leaves. The average comparative thickness of young yellow top and young normal leaves was 144 μ and 128 μ, respectively. The average thickness of mature normal leaves was 220 μ, that of sword leaves 434 μ, and of string leaves 635 μ. This increase in thickness is due to enlargement of cells of all of the leaf tissues except those of the vascular system.

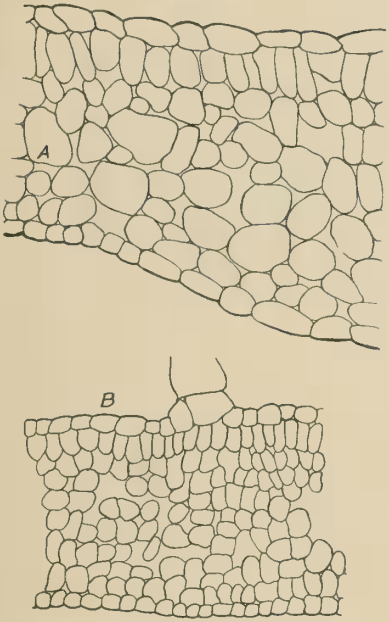

FIG. 32. Cross sections of tobacco leaves. A, young yellow leaf stage of frenching; and B, young normal leaf of the same age. The cells of the former are all hypertrophied. (Drawn by Brecher.)

The cells of frenched leaves are less well differentiated than normal, and varying degrees of inhibition of differentiation can be found. In mature string leaves all of the parenchyma may remain quite juvenile with little or no evidence of dorsiventrality. This accords with the observations of Heintzel (1900), who stated: "*Ein Querschnitt durch eines der zu rankenähnlichen gebilden um-*

geformten Blätter zeigte dass die Blattspreite nur ganz wenig entwickelt war." His study deals with mosaic, which he confused with frenching, and this statement appears to apply to frenching. Sometimes localized areas of frenched leaves will be of normal thickness, and their tissues will be well differentiated, whereas all surrounding tissues will be hypertrophied, and differentiation will have been prematurely halted. The studies of Schweitzer (1933) show that the xylem of the veins is markedly reduced in development in frenched leaves.

Effect on Chlorophyll.—Obviously, since frenching is a chlorotic type of disease, some interference with the formation of chlorophyll must occur. An effort was made, therefore, to arrive at an approximation of the extent of this interference by extraction of leaves in alcohol. Samples of equal areas of frenched and normal leaves, cut out with a cork borer, as nearly comparable as could be obtained, were extracted in a similar manner, and the extracts were compared colorimetrically. The results are shown in Table 4.

Table 4

Comparison of Chlorophyll Content of Equal Areas of Normal and Frenched Tobacco Leaves

Description of Leaves	*Chlorophyll content, Normal and frenched leaves. Ratio*
Normal and frenched young tips, grown in greenhouse	1.88 : 1.0
Large normal and frenched leaves grown in greenhouse	3.16 : 1.0
Normal and frenched young tips from field 1	2.04 : 1.0
Large normal and sword leaves from field 1	1.67 : 1.0
Normal and frenched young tips from field 2	3.51 : 1.0
Mature normal and string leaves from field 2	5.18 : 1.0

It is evident from these data that equal areas of leaf tissue of normal leaves contain from one to five times as much chlorophyll as that of frenched ones. Further examination into the problem was made by determining if this difference was due to a difference in size of chloroplasts. Measurements showed that the chloroplasts of frenched tissues are about half as large as those in normal tissues. Whether there are differences also in number of chloroplasts has not been determined.

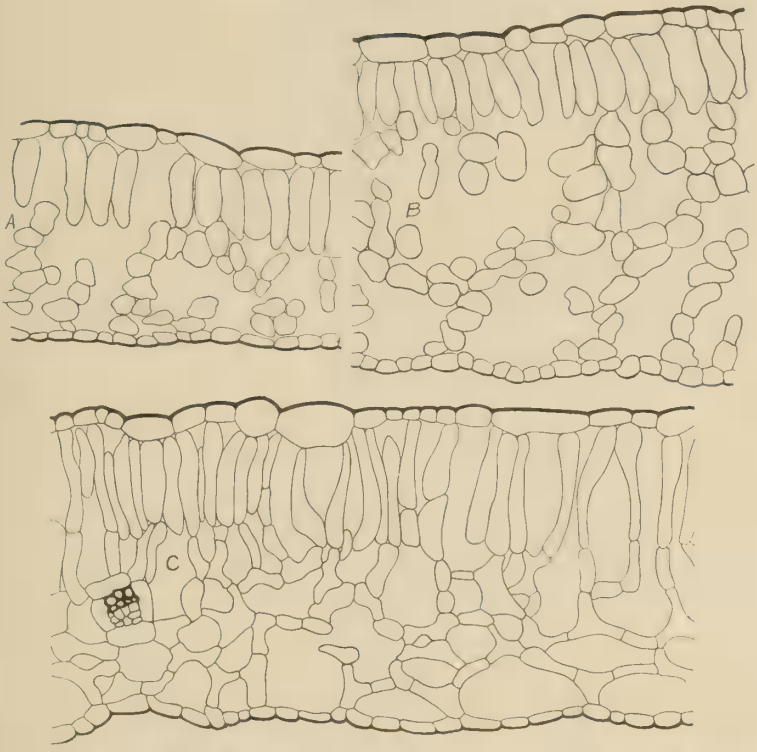

FIG. 33. Portions of tobacco leaves in cross section. A, normal mature leaf; B, string leaf; C, sword leaf. Note the increased thickness of frenched leaves, since all are drawn to the same scale. The cells of the mesophyll are enlarged and the intercellular spaces are more extensive. (Drawn by Brecher.)

PHYSIOCHEMICAL EFFECTS.—The studies on this phase of the problem involved a comparison of the water content, osmotic concentration of the cell sap, and per cent of bound water of frenched and normal tobacco leaves. The samples for these studies were collected in three localities in North Carolina and on three different dates within the period of a week. The results of these analyses for the samples from Fields 1, 2, and 3 are directly comparable with each other, those from Fields 4, 5, and 6 are likewise comparable, and those from Field 7 stand alone. The data showing differences in water content are assembled in Table 5.

TABLE 5

COMPARATIVE WATER CONTENT OF EQUAL AREAS (200 sq. cm.) OF FRENCHED AND NORMAL TOBACCO LEAVES

Field	Date of collection	Description of sample	Wet weight grams	Dry weight grams	Per cent of water	Per cent of dry substance
1	July 25	Frenched leaves	9.640	1.485	84.6	15.4
		Normal leaves	6.665	1.865	71.9	28.1
2	July 25	Frenched leaves	9.515	1.610	83.07	16.93
		Normal leaves	6.300	1.110	80.8	19.2
3	July 25	Frenched leaves	8.760	1.470	71.8	28.2
		Normal leaves	6.350	1.805	71.5	28.5
4	July 28	Frenched leaves	8.127	1.216	85.3	14.7
		Normal leaves	6.384	1.784	71.9	28.1
5	July 28	Frenched leaves	10.331	1.480	85.6	14.4
		Normal leaves	6.468	1.708	72.0	28.0
6	July 28	Frenched leaves	7.696	1.127	85.2	14.8
		Normal leaves	6.321	1.253	80.2	19.8
7	July 31	Frenched leaves	10.775	2.485	76.9	23.1
		Normal leaves	5.388	1.325	75.8	24.2
Average		Frenched leaves	9.263	1.707	81.78	18.22
		Normal leaves	6.266	1.574	74.87	25.13

The wet weight of equal areas of frenched leaves is consistently higher than that of normal leaves, the proportion being approximately 9:6. The per cent of water of frenched leaves is also consistently greater than that of normal ones, so that the proportion of dry substance in frenched and normal leaves becomes 18:25, respectively.

The osmotic concentration and bound water content of the sap of plants from the same fields were calculated from determinations of the depression of the freezing point of the expressed sap, as measured by a Beckmann thermometer.[3] These data are tabulated in Table 6.

[3] A description of the technique involved is contained in the following paper: WILSON, L. B., The effect of chlorine, bromine, and fluorine upon the tobacco plant. JOUR. AGR. RES. **46**: 889-899. illus. 1933.

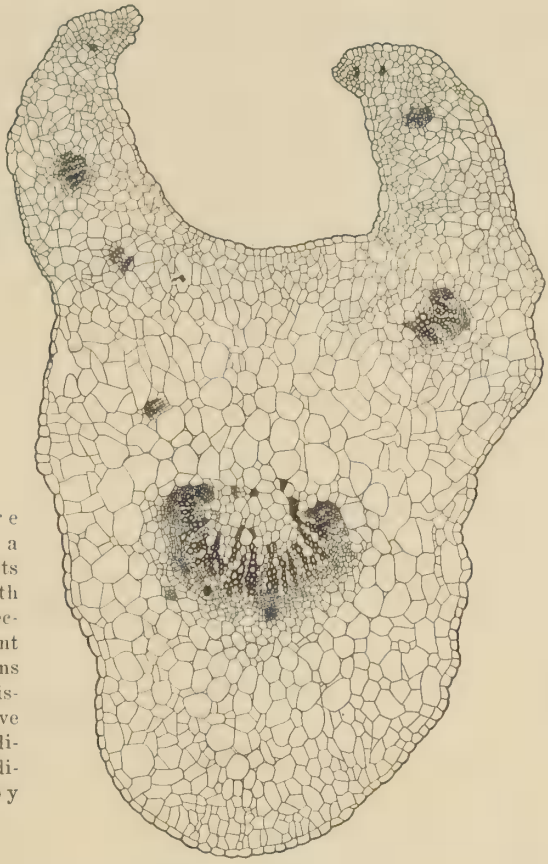

FIG. 34. Entire cross section of a string leaf. It consists mainly of midrib with narrow, lateral projections that represent the laminar portions of the blade. The tissues of the blade have remained quite rudimentary and unmodified. (Drawn by Brecher.)

TABLE 6

PHYSICOCHEMICAL PROPERTIES OF THE SAP OF FRENCHED LEAVES COMPARED WITH NORMAL LEAVES

Description of sample	Δ	Δa	Osmotic concentration in atmospheres	Per cent of bound water
Frenched leaves, field 1	.716	2.617	8.63	4.35
Frenched leaves, field 2	.698	2.593	8.40	3.52
Normal leaves, field 3	.753	2.729	9.06	5.57
Frenched leaves, field 4	.567	2.43	6.83	2.17
Normal leaves, field 5	.643	2.13	7.74	6.54
Frenched leaves, field 6	.787	3.066	9.48	7.51
Normal leaves, field 7	.891	3.16	10.73	7.12
Frenched leaves, average	.692	2.677	8.33	4.39
Normal leaves, average	.762	2.673	9.18	6.54

A comparison of the average measurements shows that the sap of normal leaves has a lower freezing-point depression, a greater osmotic concentration, and a greater per cent of bound water than that of frenched leaves. The significance of these differences is not clear at this time, but it is apparent that they could account for profound differences in metabolism.

Effect on Chemical Composition.—Use was made of chemical analyses of diseased and normal tissues in studies involving the influence of frenching upon the cellular metabolism. Samples of frenched and normal tobacco leaves, after being cured, were finely ground, dried to a

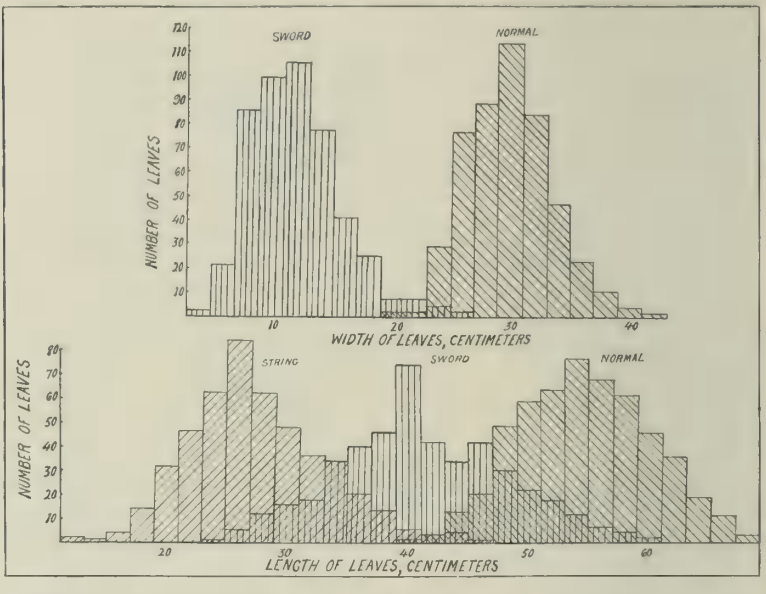

Fig. 35. Graphs representing the relative widths and lengths of approximately 500 leaves of each kind. The upper graph shows, at the left, that the range in width of sword leaves is 4 to 26 cm., nearly all of which, however, vary between 8 and 14 cm., the largest number being 12 cm. wide. The range in width of the normal leaves ranges from 20 to 42 cm., the largest number being 30 cm. wide. The lower graph shows the relative lengths of string, sword, and normal leaves. String leaves range in length from 12 to 46 cm.; sword leaves, from 24 to 60 cm.; and normal leaves, from 40 to 68 cm. Among the 500 leaves of each kind measured, the largest number of string leaves were 26 cm. long; of sword leaves, 40 cm.; and of the normal leaves, 54 cm.

constant weight, and analyzed to determine their acid, nitrogen, sugar, nicotine, and ash content.[4] Since equal areas of green frenched and green normal leaves differ in dry weight, as shown in Table 5, a correction obtained from Table 5 was applied to the figures obtained from the analyses. The results obtained from these computations of the analytical data express the percentage composition of equal areas of green frenched and green normal leaves, except in the case of the figures involving acids. These computed data are assembled in Table 7.

TABLE 7

PERCENTAGE COMPOSITION OF EQUAL AREAS OF GREEN FRENCHED AND GREEN NORMAL TOBACCO LEAVES, COMPUTED ON THE BASIS OF EQUIVALENT DRY WEIGHTS

Description of samples	String leaves	Sword leaves	Normal leaves	Sword leaves	Normal leaves	String and sword leaves	String and sword leaves	Sword leaves, tips	Normal leaves, tips	Normal leaves no fertilizer
pH	5.10	5.54	5.49	5.61	5.28	.....	5.84	5.70	5.30	5.26
Volatile water sol. acid, cc. 0.1 N alkali per gm. tobacco	.079	.042	.114	.094	.068	.423	.275	.796	.450	.214
Total non-vol acid, cc. 0.1 N alkali per gm. tobacco	15.38	20.58	14.16	20.59	15.34	16.90	20.65	17.74	15.70	16.61
Total N	2.62	1.98	1.60	1.90	1.77	3.59	2.79	3.11	2.89	2.25
Soluble N	1.40	1.46	1.08	1.39	1.24	1.56	1.26	1.49	1.60	1.24
Amino-N	.432	.368	.253	.257	.239	.599	.358	.186	.424	.314
Amide-N	.115	.072	.058	.030	.040	.132	.040	.017	.152	.113
Nicotine	1.70	3.40	2.47	3.70	3.41	1.37	1 59	3.11	2.79	2.46
Reducing sugar	5.24	7.23	15.02	6.79	16.83	4.01	3.18	3.60	7.27	11.06
Total sugar	5.38	7.29	15.09	6.95	17.11	4.05	3.20	3.62	7.68	11.70
Petroleum ether extract	4.08	5.62	5.59	5.98	6.79	3.22	4.97	5.98	4.57	4.05
Silica-free ash	10.16	10.69	11.17	11.34	8.71	.....	.....	.....	.....	.....
CaO	2.85	4.25	3.97	4.56	3.33	.....	4.50	3.32	3.36	.....
K_2O	3.10	2.18	2.21	1.85	1.74	.....	.....	.....	.....	.....

The samples from which the comparable data in columns 1, 2, and 3 were obtained, were collected in the season of 1930; the remainder were collected in 1931. The results in columns 4 and 5 are comparable, likewise those in columns 6 and 7, and those in columns 8 and 9.

An examination of these data reveals that the total

[4] The analyses were made according to official methods at the Tobacco Research Laboratories, Duke University.

acid content of frenched leaves is higher than that of normal ones and is correlated with a lower sugar and a lower ash content. Another outstanding difference is the higher total nitrogen and nicotine content of the frenched leaves.

If an attempt is made to picture the distribution of these constituents within the green leaf, the results are quite different from those in Table 7. In this case it must be recalled that frenched leaves are about twice as thick as normal leaves. Since the analytical results from the chemical analyses of frenched and normal leaves were based upon equivalent dry weights, the data were recalculated so as to express the composition on the basis of equal volumes of frenched green and normal green leaves. These recalculated data are assembled in Table 8.

It is apparent from these data that equal volumes of frenched leaves have less total acid, nitrogen, nicotine, sugar, and ash than normal ones, which might be inter-

TABLE 8

PERCENTAGE COMPOSITION OF EQUAL VOLUMES OF GREEN FRENCHED AND GREEN NORMAL TOBACCO LEAVES, COMPUTED ON BASIS OF EQUIVALENT DRY WEIGHTS

Description of samples	String leaves	Sword leaves	Normal leaves	Sword leaves	Normal leaves	String and sword leaves cured on stalk, not split	String and sword leaves cured on stalk, split	Sword leaves tips	Normal leaves tips	Normal leaves no fertilizer
pH	5.10	5.54	5.49	5.61	5.28	.....	5.84	5.70	5.30	5.26
Volatile water sol. acid, cc. 0.1 N alkali per gm. tobacco	.042	.021	.114	.047	.034	.215	.138	.400	.450	.214
Total non-vol acid, cc. 0.1 N alkali per gm. tobacco	7.83	10.48	14.16	10.49	7.85	8.45	10.33	8.88	15.70	16.61
Total N	1.33	1.08	1.60	.96	1.77	1.80	1.40	1.56	2.89	2.25
Soluble N	.613	.744	1.08	.708	1.24	.78	.64	.75	1.60	1.24
Amino-N	.220	.187	.253	.130	.239	.299	.179	.097	.424	.314
Amide-N	.058	.037	.058	.015	.040	.066	.021	.009	.152	.113
Nicotine	.866	1.73	2.47	1.88	3.41	.69	.80	1.56	2.79	2.46
Reducing sugar	2.67	3.68	15.02	3.56	16.88	2.00	1.59	1.80	7.27	11.06
Total sugar	2.74	3.71	15.09	3.46	17.11	2.03	1.60	1.81	7.68	11.70
Petroleum ether extract	2.08	2.86	5.59	3.04	6.79	1.61	2.49	2.99	4.57	4.05
Silica-free ash	5.17	5.44	11.17	5.77	8.71	.....	.....	.....	.....	.....
CaO	1.45	2.16	3.97	2.30	3.33	.....	2.25	1.66	3.36	.....
K_2O	1.58	1.11	2.21	.924	1.74	.....	.....	.....	.....	.....

preted to mean that all metabolic processes within the leaf are retarded. The data on the relationship of calcium and potassium in the ash accord with those of Haas, Batchelor, and Thomas (1928), who found that the ash of walnut—affected with little-leaf, a disease of the same type—is lower in calcium and higher in potassium than that of healthy leaves.

The chemical analyses of Schweitzer (1933) show a higher content of potassium and nitrogen in frenched than in normal leaves, with from two to three times as much iron in diseased as in normal plants. Other data, not herein included by the writer from analyses of entire plants, stems, and leaves do not show the absence of any element in diseased tissues and its presence in normal tissues, nor can the difference in amounts of any elements be employed in arriving at an explanation of the cause of frenching.

Cause.—The cause of frenching has not been unequivocally determined. A number of investigators have maintained that the disease is associated with such predisposing factors as excessive moisture, defective drainage, deficiencies of certain mineral elements, soil reaction, or soluble toxic substances, and some have been able to correct frenching, but no one has been able to produce the disorder at will, repeatedly, and under controlled conditions.

Evidence of Non-infectiousness.—Clinton (1914) expressed the juice from leaves of the string leaf phase of frenching and inoculated therewith fifteen plants growing in crocks in the greenhouse. No infection occurred, but one plant became mosaic from an unknown source of inoculum. He was at that time investigating mosaic and was aware of the fact that it was distinct from frenching, as shown by his statement (Clinton, 1914) that "string leaf is a malformation of the leaves that sometimes accompanies calico (mosaic), but is not dependent upon the calicoed condition for its development."

Wolf and Lehman (1924) grafted buds from frenched plants onto normal tobacco with the result that the buds

recovered and the stocks remained normal. Valleau and Johnson (1927) on three occasions grafted frenched stem tips on healthy plants, and in each case the tips recovered. The writer has on several occasions removed frenched plants from the field and set them in other fields among healthy plants. Following transplanting, the new growth on these plants was in all cases normal. When healthy plants were transplanted into the positions from which the frenched ones had been removed, they rapidly became frenched. Garner (1922) expressed the opinion that frenching is non-infectious. No one has been able to communicate frenching from one plant to another nor to secure any evidence that the disorder is infectious.

Evidence of a Water Relationship.—The evidence of a relationship of moisture to frenching is largely observational, and growers have long believed this relation to exist. The report by Dodge (1874), previously quoted, states that "Too much wet weather after the plant starts to grow . . . [causes] it to french. . . ." The observations of Wolf and Moss (1919) led them to conclude that "Excessive soil moisture, due to a wet season, proximity to the water table, to ditches, to stumps, or to small basins where water may collect, is very commonly associated with the disorder." In a field that he had under observation, Shear (1933) noted that frenching first appeared in mid-August in 1931, following a heavy rain that fell about two weeks earlier. Rainfall had been deficient during the early part of that growing season. The total rainfall in August was 7.28 inches, and toward the end of the season frenching became increasingly more severe. The rainfall during May and June of the following year, however, was excessive, and by the last week in June over half of the tobacco plants grown on this same area were frenched.

During 1931 the writer saw a field of tobacco of about five acres in which the yellow top form of frenching appeared on the entire crop following a heavy rain. The plants had entirely recovered after ten days. Frenching reappeared and disappeared twice subsequently in this

same crop. Each recurrence was apparent about three to four days after a heavy rain.

On February 23, 1930, tobacco plants were potted in soil in which tobacco was known to french. The soil for a portion of the series was kept saturated, another portion was supplied with sufficient water to provide optimum conditions for growth, and a third was watered only enough to prevent wilting of the tobacco plants. By March 17 all plants in the series had frenched, with no appreciable difference except that the frenching was apparent first on the plants in the wet soil and last on those in the dry soil. Apparently, excess of water is not the primary cause of frenching, but is to be regarded as a contributing factor.

Evidence of a Nitrogen Relationship.—Valleau and Johnson (1926, 1927) interpreted their investigations to show that the frenching problem is one of a nitrogen deficiency. They supplied potted frenched tobacco plants with a variety of nitrogenous materials and brought about the recovery of these plants with each of these materials. They properly raised the question whether recovery is due to the direct effect of the nitrogen compounds on the plant or whether it is a secondary one resulting from the action of the nitrogen compounds upon the soil. In answer to this query they supplied nitrate to frenched tobacco plants without having it enter through the base of the plant. This was done by bending over two plants; and when the growing points were again directed upward, the bends were tied down in sand where they rooted. Nitrate was then supplied through the roots at the bend of one of the pair of plants, and distilled water to the other. In the case of the former, prompt recovery was effected, while the latter remained frenched. This shows that frenched plants may recover following the absorption of inorganic nitrogen compounds, but cannot be taken to prove that frenching is due to a deficiency of such compounds.

Shear (1933) attempted to correct frenching by applying ammonium nitrate to plants placed over beakers

containing a .02 per cent solution of this salt. Some of the roots extended through the hole in the bottom of the pot and into the solution in the beaker below. No recovery occurred, whereas frenching was corrected in two weeks when the nitrate of the same concentration was applied to the soil. The writer has employed the same method of application of salts, that is, through absorption by exposed roots, and effected the recovery of frenched plants with sodium nitrate, ammonium sulphate, calcium nitrate, manganese sulphate, manganese chloride, and manganese acetate.

In field trials Shear's (1933) data show that nitrogenous fertilizers were ineffective in preventing frenching. Hopkins (1930) claims, however, that frenching was corrected in South Africa by the application of 50 to 100 pounds of nitrate of soda per acre. Haas (1929) corrected frenching in potted plants by the application of nitrates. Further evidence that frenching is not primarily due to a deficiency of nitrogen is afforded from twenty year's observations of the nitrogen fertilizer plots at the Tobacco Experiment Station, Oxford, N. C. It may appear in plots to which no nitrogen is applied, in those given little nitrogen, normal applications, or excessive applications.

Evidence of a Lime Relationship.—Three striking cases have come to the writer's attention in which severely frenched crops developed in fields to which a ton or more of ground limestone per acre had been applied, whereas normal crops had always been grown in these same fields in previous years. In another case, in 1930, frenching appeared on the sucker growth of the fertilizer plots, at the Tobacco Experiment Station, Oxford, N. C., to which lime had been applied. Two limed strips extended across the rows in all plots, and frenching was confined to the plants in these limed strips. These observations agree with those of Karraker and Valleau (1930), and indicate a relationship of calcium to frenching. Attempts were made to learn something of this relationship by a series of experiments in which different

calcium salts were applied to soils in pots in which frenched plants were growing. Those to which calcium nitrate, calcium chloride, calcium permanganate, and calcium acetate were applied recovered rapidly. No benefit followed the application of any one of the ten other calcium compounds that were applied in a similar manner. When, however, calcium nitrate in dilute solution was permitted to be absorbed through exposed roots of frenched tobacco plants, they quickly recovered, whereas no recovery occurred with calcium chloride similarly applied, even though it was supplied for a month or more. The only conclusion that is indicated from the apparently inconsistent results is that frenching does not appear to be related to calcium as a primary cause. This experimental procedure, moreover, as applied to frenching, is open to criticism since the correction of a disorder by the application of an element or compound does not postulate that this same disorder will arise if this element or compound is withheld.

Evidence of a Relationship with Soil Reaction.—A relationship between the occurrence of frenching, the soil reaction, and the availability of nutrients was found from the work of Karraker and Bortner (1934). The disease did not develop on soils of moderate or strong acidity irrespective of the supply of nutrients. In less acid soils, however, it developed when the amount of nitrogen, potassium, or phosphorus, singly or in combination, available for the tobacco plants was small, but the disease did not develop when an abundant supply of these nutrient elements was available. Sweitzer (1933) reported that frenching soils in Java were weakly alkaline, and that their total and available supply of nitrogen was low. Shear (1933) determined the pH of soil around frenched plants, found it to range from 5.2 to 7.1, and states that in his field experiments frenching developed more slowly on the more acid soils. In soils in which he adjusted the reaction prior to setting the plants, he found that the disease develops most rapidly between pH 7.0 and 7.55. The writer's observations of the pH of soils around frenched

plants, made over a period of five years, show a range of pH from 6.8 to 7.8. Frenched plants in pots, in soil whose reaction was pH 7.4, were used in a series of experiments to test the influence of soil reaction. Either sulphuric, nitric, acetic, or citric acid in dilute solution was applied. After ten days to two weeks the plants in all pots to which acids had been applied were normal; and the check plants, given tap water, remained frenched. The reaction of the soil around the recovered plants was at this time approximately pH 5.4. The subsequent growth continued to be normal for a period of four to five weeks when the unfolding leaves were again affected. Further addition of dilute acid was ineffective in correcting the frenching. This experiment was repeated twice subsequently with similar results. The recovery of plants from frenching in these experiments was not due directly to the effect of acids as indicated from another series of experiments in which potted frenched plants were placed upon glasses containing acids diluted to approximately pH 5.4. A portion of the roots extended from the holes in the bottom of the pots into the dilute acids in the glasses below. These plants exhibited no evidence of recovery even after several weeks of exposure to conditions that permitted them to absorb the acids.

Further evidence of an indirect beneficial effect of acid was secured as the result of the application of sulphur to frenching soil in fields. In one typical case the sulphur was applied to a small area twelve months prior to the time that tobacco was transplanted in this field. The plants in the sulphured area remained normal, but those on the surrounding area were all frenched. The soil reaction in the sulphured area was pH 5.6 and that of the surrounding soil was pH 7.2.

Effect of Sterilization of Frenching Soils.—Valleau and Johnson (1927) partly sterilized frenching soil by heating in an autoclave for a brief period at 65° to 75° C., with the result that one of the eighteen plants set in the heated soil frenched. Shear (1933) autoclaved frenching soil at 15 to 18 pounds pressure for fifteen min-

utes, and plants grown in this soil did not frenchi. Dr. Brecher autoclaved frenching soil, one portion for ten minutes, another for twenty minutes, and a third for forty minutes. All tobacco plants grown in the first lot frenched early, two of the four in the next lot frenched somewhat later, and one only in the last lot frenched after two months.

Evidence of a Toxicity.—In studies with tobacco grown in solution cultures containing one part per million of thallium, applied as nitrate, McMurtrey (1932) noted that a series of symptoms, including chlorosis and distortion of the leaves and proliferation of the lateral buds, occurred. These symptoms are like those of typical frenching, but he did not conclude that thallium toxicity and frenching are identical. The writer has been able to verify these results when he employed thallium nitrate, but was unable to do so with thallium sulphate.

Shear (1933) watered four plants in sand culture with leachings from a frenching soil. After a month frenching developed in one, and two weeks later, in another. The control plants, watered with distilled water, remained normal. This result, he concludes, indicates that frenching is the result of some toxic material in the soil. The writer has made similar studies on three frenching soils, but without the development of a single frenched plant among those watered with the leachate. At least a score of plants among the controls, watered with distilled water, developed frenching. No reason can be assigned at this time for these contradictory results. That they may be expected is indicated by the fact that three sandy, frenching soils, when stored in the greenhouse for a few months, lost their ability to cause frenching. Two clayey frenching soils, however, were equally as potent in producing frenching even after a year's storage. Schweitzer (1933) observed that frenching soils dried in the sunlight lost their ability to induce frenching or the plants were only slightly affected, which he interprets to indicate that the cause is a toxin.

Discussion of Evidence on the Cause of Frenching. —Even though the primary cause of frenching is not yet known, it seems certain that the disease is non-infectious. Evidence in support of this conclusion rests (1) upon inability to communicate frenching from diseased to healthy plants, (2) upon the recovery of frenched plants when transplanted into other situations, and (3) upon the recurrence of frenching upon soils that have been sterilized with steam. The only evidence that the cause of frenching may be an infectious agent is that of Severin (1929). He produced a condition that resembles frenching following transmission from beets to tobacco of the virus from beets affected with curly top, through the agency of beet leaf hoppers.

That frenching is due to some soil factor rests upon the observation of its recurrence year after year in the same sites. This factor is not moisture alone because the disease occurs whenever the frenching soil contains sufficient moisture to maintain the growth of the tobacco plant. Excessive moisture merely aggravates the condition.

Frenching is not due to the physical characteristics of the soil. This conclusion is supported by the fact that the disease occurs in all sections where tobacco is grown and hence on a variety of soil types. When frenching soil is screened, furthermore, the portion that passes through the finest-meshed soil sieve is as capable of producing frenching as that retained on the coarsest sieve.

This disorder is not produced by lime per se, although it is favored by soil alkalinity and is aggravated by liming. This is indicated by recovery of frenched plants when the soil is acidulated, but by failure of the plants to recover when acids are applied, to growing plants, through exposed roots. It is further indicated by the fact that affected plants recover when certain calcium salts are applied to the soil, but no beneficial effect is evident when some of these same salts, calcium chloride, for example, are applied through the exposed roots.

Frenching of tobacco does not seem to be caused by

a toxic material within the soil, either inorganic or organic. If it were an inorganic substance, the fact that frenching occurs so widely throughout the world would indicate that it must be a compound of some rather common element. Again a toxic inorganic substance could be expected to affect crops other than tobacco, especially solanaceous crops, when these crops are grown in frenching soils. Frenching of other crops is not known to occur except in the case of petunias and tomatoes. It may develop that the cause of frenching of tobacco is identical with that of little leaf of walnut, mottling of *Citrus*, and little leaf of peach and apricot, all of which troubles are brought about when these kinds of trees are planted side by side on the same soil (Haas, Batchelor, and Thomas, 1928).

If the cause of frenching were an organic material, it should disappear from the soil through decomposition induced by microörganisms within those soils. Instead, it recurs on every tobacco crop grown on certain areas, even though intervals of three or four years exist between successive crops of tobacco. This statement is based on the observations of the "rotation plots" at the Tobacco Experiment Station, Oxford, N. C., where frenching always appears when certain plots are planted with tobacco.

Again, if frenching were due to a toxic material in the soil, it should appear in the leachate. The evidence that the leachate, when applied to tobacco, will cause frenching is as yet both meager and contradictory.

Frenching does not appear to be caused by the deficiency of any specific single element. It occurs in crops to which "complete mixed fertilizers" have been applied. The evidence to date from studies on the effect of a specific element are inconclusive, either because the element was applied to such heterogeneous substrata as to soils, or to water cultures containing mixtures of mineral elements. Relationship of the element to frenching has been conditioned, in these experiments, upon the recovery of frenched plants. Such evidence is not trustworthy.

When, under controlled conditions, frenching can be produced at will by withholding a single mineral element, and affected plants can be made to recover by supplying this element, then and only then can the relationship of any specific element to frenching be established.

The most plausible explanation of the cause of frenching would appear to be that alkalinity interferes not only with the availability, but also with the rate of absorption of the mineral bases by the tobacco plant, and possibly, by effects within the tobacco plant, impedes their utilization.

Control.—Until the cause of frenching has been determined no rational recommendations for control can be given. The occurrence of the disease following the application of lime, and the observations that frenching soils are usually alkaline or weakly acid, indicate that lime should be used with caution. They furthermore indicate that acid-forming materials like sulphur may have value. Ammonium sulphate might be expected to be beneficial if used as one of the sources of nitrogen.

Frenching soils are generally low in humus. There is evidence that the application of compost or of manure tends to cause plants grown on frenching soils to remain free from frenching.

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Green-Spot or Verderame

Attention was called (Splendore, 1911) to the presence of green spots on cured leaves of tobacco in Italy. It manifested itself as isolated or as more or less grouped or confluent green areas. The edges, tips, and petioles of the leaves were free from these defects, but they might occur on the middle portions of the blades. It appeared to be most prevalent on oriental varieties of tobacco. Splendore applied to this condition the names *verderame* and *bassarà* and was unable to determine the cause.

Lo Priore (1916, 1921) noted the same disorder on both oriental and occidental tobaccos in Italy, but was likewise unable to determine the cause of the formation of the green spots. He ventured the opinion that it might be due to some soil condition, or might even be due to a virus. His experiments showed the presence of more starch in the green areas than in the surrounding tissues.

Apparently the same disorder was described by Scherffius (1924) on Turkish tobacco in South Africa, as shown by the colored plates that accompany his report.

He found that the trouble "is caused by harvesting with gummy hands the tobacco in the heat of the day, and bruising the leaves in the operation." The writer has observed greenish spots, apparently of the same type, on flue-cured tobacco that was bruised in harvesting. These spots disappear, however, while the cured leaves are stacked in the packing house preparatory to being graded.

It may be of interest, in this same connection, to call attention to "oil-spot" or "green-spot" on lemons, oranges, and grapefruits that are bruised when picked while the rind is still green (Fawcett and Lee, 1926), since it is undoubtedly the same type of disorder.

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LIGHTNING INJURY

Brief mention was made by Wolf and Moss (1919), under the names of "crook-neck" or "limber-neck," of a disorder of tobacco whose cause was at that time unknown. Subsequently Clinton (1920) described a similar condition that he had observed in Connecticut, and properly attributed it to lightning injury.

Injury from lightning involves plants in more or less circular patches. The plants at the centers of these areas are killed and those toward the margin may show any of the following symptoms. A broad, brown streak may extend from the soil level upward along one side of the stem into the midribs of the leaves. The stems may be injured and the midribs be collapsed, beginning at the leaf tips. The leaves on one side of the plant may be killed or partly collapsed, and those on the other may be quite normal. Some leaves may be brown and dry at the tips and along

the midveins. If the plants are seen a week or two after the lightning stroke, the continued growth of the uninjured side and its failure to elongate on the injured side may cause the stem tip to be turned downward or to have twisted to make a complete loop. Likewise the uneven growth of the injured midrib and the normal leaf blade may have resulted in the leaves becoming puckered and distorted.

Clinton (1920) described a second type of injury caused by lightning. This may occur in shade-grown tobacco when the cloth is ignited by the bolt. The heat from the flaming cloth or the falling flaming fragments of cloth may cause considerable injury to the tobacco beneath.

It appears probable to the writer that *zwarte popoe ziekte* (black bud disease), described by Jensen (1919), may be lightning injury.

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FIG. 36. The killing of plants in this circular patch is the result of a stroke of lightning. (From James Johnson.)

Frost Injury

Injury to seedlings from frost is of very common occurrence in the flue-cured-tobacco district. When frost forms on the seed-bed covers, brown dead areas appear after a day or two on the portions of the leaves that were in contact with the covers. This is of itself of little consequence, except that the presence of the dead leaf tips may cause the grower to become unduly alarmed in case he is not aware of the cause. The tissues that have been killed may, in time, fall away, and, in consequence, the leaves are notched and otherwise misshapen.

Clinton (1920) described a mottling of tobacco in Connecticut that he attributed to frost. The affected foliage showed small, white specks, closely aggregated, or else large white spots. Obviously light frosts had destroyed the chlorophyll in the areas that subsequently became whitened.

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Hail Injury

Damage to tobacco from hail usually involves only relatively small areas. The crops included in the path of the storm, however, may be completely ruined. If hail occurs after the tobacco plants have attained their mature size, the leaves may be beaten off or be so badly shredded that they are practically worthless. Brown, irregular areas, that become sunken and grayish, mark the places where the hail stones struck the stems. If the crop is young, it may largely recover provided the storm was not too severe.

Marbling

Little is known regarding a disorder that has been reported from Sumatra (Jochems, 1926) as *marmer*, the Dutch word for marbling. The natives of Java call it *brontong*. The writer has observed, in North Carolina, what he regards as this same disease. It appears as marbled irregular areas in which winding lines of white and

yellow, or else pale green or yellowish green, form fantastic patterns. Its cause is entirely unknown, and there is no evidence that it is infectious. Jochems was not able to correlate its presence with soil types or with weather conditions. He noted that in some cases it occurred on the leaf tips or on leaves exposed to the sun. In other cases, however, the upper leaves of an affected plant were normal, and the shaded leaves were marbled.

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ALBINISM

Albinism in plants is a phenomenon with which everyone is more or less familiar, having seen it in such plants as geranium *(Pelargonium)*, *Evonymus*, ivy *(Hedera)*, and wandering Jew *(Zebrina)*. Horticulturists and gardeners have for a long time propagated and sold albinistic or variegated plants for their ornamental value. The cause of albinism is not established, but it is not infectious, and, in the case of some species, it is heritable.

Albinism in tobacco has been reported in several countries under such names as *panaschierung, hellfleckigkeit, panachure, boudblad, albinismus*, or *albicatio*. It is not unusual to find on the foliage of occasional tobacco plants large, irregular patches that are cream white, yellowish green, or greenish yellow, and that give to the leaves a marbled appearance. The patches are limited by the veins, and the colors may either be the same on both leaf surfaces or be quite different.

Microscopic examination shows that the color differences are due to a differential involvement of the chlorophyll of the tissues of the leaf. If both the palisade and spongy parenchyma are involved, the areas are cream white or yellowish white. If the spongy tissue is normal and the palisade tissue is affected, the leaves are yellowish green. Pantanelli (1905) found that variegated foliage contains more enzymes, including oxidase, perox-

FIG. 37. Lightning injury to a leaf. The leaf is twisted, and the tip and much of the midrib are dead.

FIG. 38. Patterns of color of different intensities of yellow and green. The lines are contoured, producing a condition termed marbling whose cause is unknown. Apparently a form of mosaic.

FIG. 39. Albinism of tobacco leaves. The color of the light portions varies from creamy white to yellow and yellowish green. The dark portions are normal.

FIG. 40. Two tobacco leaves, one viewed from above and the other from below, that show a peculiar deformity. The basal portions are broad, and the tips are slender and tapering. The leaf margins and tips are curled downward and the veins are close together. The cause of this disorder is unknown.

idase, diastase, and protein-splitting enzymes, than normal tissues. He also found that the chloroplasts are either smaller or less numerous, or that both of these hypoplastic symptoms might occur. In some cases the affected tissues are less differentiated than normal ones.

Seed from albinistic tobacco is known to give rise to affected plants in small proportion.

Reference

Pantanelli, E., Studii su l'albinismus nel regno vegetale (Studies of albinism in plants). Malpighia **19**: 45-63. 1905.

Injury from Tarvia

It has long been known that the fumes from tarvia, used largely for surfacing roads, are injurious to vegetation. A brief summary of the investigations dealing with this matter is contained in the studies by Chivers (1917). In general, these investigations show that the injury is produced by certain volatile products, notably phenol and pyridine, contained in tarvia, and that species of plants differ in their susceptibility to injury. The extent of this injury may vary from shrivelling and drying of the leaves and complete death of the plants to slight injury manifested by small lesions or slight modification of the normal color.

Injury to tobacco from tarvia fumes has been reported from Connecticut. Plants nearest the road that was being surfaced had brown lesions that involved the intercostal tissues and leaf margins. The leaves of those more distant had a glistening, varnished appearance. Manifestly the concentration of the fumes to which the tobacco plants are exposed is the factor which determines the nature or extent of the injury to the foliage.

Reference

Chivers, A. H., The injurious effects of tarvia fumes on vegetation. Phytopath. **7**: 32-36. 1917.

Sun Burn

When the leaves of tobacco are turned by the wind so that the lower surface is exposed to direct illumination, large, irregular, dead areas may form. This condition

may be termed sun burn or sun scorch. When heavy winds occur during harvest, this disorder becomes rather common. Sun burn may also appear when particles of sand are splashed on the leaves by rains. These particles may become heated to the extent of causing small brown specks to appear.

Leaves that are mosaic are especially prone to develop necrotic spots during periods of intense illumination, and, in this case, the injury is merely aggravated by exposure to bright sunlight.

Leaf-Drop

The name "tobacco leaf-drop" was applied by Wooliams (1928) to a peculiar disorder that he first noted in British Columbia in 1927. It usually appears when the crop is nearly ready to be harvested. At first only an occasional plant is involved, but the condition rapidly spreads throughout the field. The disease is manifest by the shedding of the leaves, beginning with the lowermost leaves and advancing upward. The petioles break off about an inch from the point of attachment of the leaves. In some cases only one or two of the lowermost leaves are shed, and in others the shedding may continue until the plants are completely defoliated. This strange condition is not known to occur elsewhere, its cause has not been determined, and Wooliams was unable to find any pathogenic agent associated with affected plants.

Reference

Wooliams, G. E., Tobacco diseases in Summerland, British Columbia. Report of the Dominion Botanist for 1927. Div. Bot. Canada Dept. Agr. 226-228. 1928.

Club-Root

Brief mention of the presence of a disease in Kentucky called club-root was made by Valleau and Johnson (1932). The roots of affected plants are very much thickened and knotted, simulating the appearance of the roots of cabbage and related plants when affected with the club-root disease. The lower leaves of affected plants are normal, and those formed thereafter are pro-

gressively more and more dwarfed. The elongation of the stem is inhibited so that the entire plant is low-growing or dwarfed in appearance. The cause is entirely unknown, except that it is entirely distinct from root knot, caused by nematodes, with which it has in the past been confused. The disease does not appear to have been noted in any other tobacco-growing area.

REFERENCE

VALLEAU, W. D., and JOHNSON, E. M., Tobacco diseases in Kentucky. KY. AGR. EXP. STA. BUL. **328**: 109-150, illus. 1932.

MONSTROSITIES

From time to time note has been made of malformations or deformities of tobacco of non-parasitic origin. These are sometimes called teratologies, and while they are of interest, they are of no economic importance. A summary of these observations on teratologies of tobacco has been made by Penzig (1922).

Among the early accounts of this nature is that of Bischoff (1839) in which he describes the fusion along the midribs of two leaves of *Nicotiana macrophylla*. A similar condition has been reported in *N. tabacum* by Fermond (1857) and Jochems (1926). This type of concrescence appears to be of more rare occurrence than that of fusion of stems which results in the formation of flattened stems, a condition called fasciation. Fasciation of Samsun, a Turkish variety of tobacco, was described by Paolini (1907).

Fermond (1857) noted that three cotyledons may occur on occasional seedlings of *Nicotiana tabacum*, *N. rustica*, and *N. paniculata*. In the case of some of these seedlings a whorl of three leaves developed just above the cotyledons, at the next node there were two leaves opposite each other, and the leaves on nodes that formed subsequently were normally arranged.

The occurrence of leaves in which the midvein is branched and the leaf, in consequence, has two or three tips has been noticed by many. Trifid tipped leaves were described by Fermond (1857), and forked tipped ones, by Buchenau (1888) and by Jochems (1926).

The entire leaf may be beaker-shaped, as observed long ago by Kickx (1863), or a beaker-shaped secondary leaf may arise from the midrib, as illustrated and described by Buchenau (1888) and by Jochems (1926).

Instead of the normal inflorescence the flowers may consist of a cluster of small green leaves. Greening of the flowers, called virescence, was reported for *Nicotiana*

FIG. 41. Malformations or teratologies of tobacco. Nos. 1, 2, 3, 4, and 5 show buds arising from the axils of the floral leaves. No. 11, a single set of calyx lobes investing an opened flower and a bud. No. 12, calyx of 6 irregular sepals subtending an unopened corolla. No. 13, flower consisting of 6 sepals and a rudimentary bud which replaces all of the other flower parts. Nos. 14 and 15, flowers with green corollas. (After Ghimpu.)

rustica by Moquin-Tandon (1841), Fermond (1857), Savelli (1920), and more recently by Ghimpu (1931). The last-named writer has made an extensive study of abnormalities, especially of the floral organs. He listed and illustrated thirty-two monstrosities that he observed under natural conditions in Rumania, and sixteen that he induced experimentally. Among these may be noted (1) an increase in the number of parts of one or more of the organs of the flower; (2) a decrease in the number of parts of one or more of the organs of the flowers; (3) proliferation of the flower, resulting in a flower within

FIG. 42. Malformations of tobacco. No. 1, green corolla, two slender leaves at center of flower. No. 2, calyx of 2 small and 2 large sepals with corolla of 2 green leaves. Nos. 3 and 4, flowers in which the organs are green and leaf-like. No. 5, corolla of small petioles that are partly green, the receptacle bearing at the center three leaves and a floral bud. Nos. 6 and 7, three-carpelled ovaries. Nos. 8 and 9, calyx with three separate sepals, having 2 floral buds at the center enveloped in one-corolla. No. 10, synanthy. No. 11, opened corolla enclosing 2 ovaries borne on a double stalk. No. 12, endogenous inflorescences. No. 13, two fused peduncles bearing 3 flowers. Nos. 14 and 15, syncarpy. No. 16, stalked corolla. No. 17, torsion of flower. No. 18, hypertrophy of stamens and pistil. No. 19, hypertrophy of pistil and torsion of flower. No. 20, leaf that is narrow and curved. No. 21, abortion of one side of the leaf of *Nicotiana glauca*. (After Ghimpu.)

a flower; (4) virescence or greening of organs other than the calyx; (5) calyx and corolla united to form a perianth; (6) stamens united; (7) multiple ovaries that are united; (8) ovary borne on a petiole; (9) stamens with one normal anther and the other petaloid; and (10) the calyx of separate members, all of which are large and leaf-like.

Among the abnormalities in flower structure that have been recorded in other accounts are the occurrence of hexamerous flowers with three carpels instead of four, of flowers with epipetalous carpels, or of flowers that were zygomorphic and two lipped like those of the *Scrophulariaceae*, and in which one of the stamens was abortive (Penzig, 1922). Fermond (1857) observed a case, in *Nicotiana rustica*, of five complete flowers in one and the same calyx. In *N. sylvestris*, Savelli (1920), saw ovules that were modified into small carpels, and also other abnormalities (1933). Westhoff (1879) reported the presence, in *N. tabacum*, of flowers each of whose petals was rolled into a tube on which a stamen was inserted. Costerus (1907) and White (1913) noted cases in which the stamens became petals or pistils.

The significance of malformations and the inheritance of certain kinds of teratologies have been considered by Fermond (1872) and by White (1916), to whose accounts the student of this problem is referred.

The occurrence of a condition called gigantism, that appears properly to be regarded as a monstrosity, has been noted by Allard (1919) in Maryland Broadleaf tobacco. He found that the giant plants that appear spontaneously in this variety may bear three to four times as many leaves as the ordinary plants and that this gigantism is a heritable character. This type of monstrosity is, therefore, a useful one.

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FIG. 43. Fasciated stems of tobacco. (From Nakata.)

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VI

Diseases of Tobacco Caused by Viruses

Nature of Viruses

The word *virus*, as first employed, meant poison. To the biologist it has come to have another meaning that is entirely different. The publication by Mayer (1886) of his observations on mosaic of tobacco marks the beginning of our knowledge of diseases of plants of virus origin. Subsequently over two hundred species of plants occurring in widely different families have been recognized as being affected with diseases of this type. As might be anticipated, a conflict of opinions and observations has resulted from the explorations in this field of plant pathology. The work of certain investigators indicates that one virus is concerned with mosaics on a wide variety of unrelated plants, as has been shown to be the case with the virus of tobacco ring spot, of aster yellows, of beet curly top, and of tomato-spotted wilt. That of others shows that the infective agent may be restricted to a single species or to a few closely related ones. It follows from the conclusions of this latter group of investigators that there is a group of specifically distinct viruses, each of which is capable of causing a distinct mosaic disease.

Virus diseases include those disorders whose causes are ultramicroscopic, filterable, infectious principles or agents that are non-cultivable except in living tissues. Such an arbitrary definition is obviously subject to criticism because of the exceptions that immediately arise in the mind of anyone who has been interested in the problem of the nature of viruses. Whether a virus will pass through a clay filter, one made of diatomaceous earth, or a collodion membrane will be determined, in

part, by the size of the openings in the filter or by the grade of the filter; and in part, by the hydrogen ion concentration of the liquid to be filtered. Again, recent investigations have apparently demonstrated that the viruses which cause certain animal diseases as vaccinia, pleuropneumonia of cattle, and laryngo-tracheitis of fowls, can be cultivated apart from living cells.

According to Rivers (1932 b), who also defines virus diseases in terms of the characteristics of the causal agencies, viruses possess the following properties: (1) they are capable of multiplication only in the presence of susceptible cells; (2) they produce disease, in many instances, in a single species of host; (3) the presence of inclusion bodies within the cells of invaded tissues is one of the evidences of virus diseases; and (4) lasting immunity, especially in the case of virus diseases of animals, usually follows attack. It may be stated in criticism of this definition, facts of which Rivers was aware, that several plant diseases of virus origin, as ring spot of tobacco, ordinary mosaic of tobacco, spotted wilt of tomato, and aster yellows, have a wide suscept range. In addition, immunity in the case of plant diseases caused by viruses is not known to occur, with the possible exception of tobacco ring spot. In diseases of animals, the immunity following such diseases as common colds, influenza, and foot and mouth diseases is only temporary. In the case, however, of such diseases as small pox, measles, and chicken pox, immunity lasts throughout the life of the individual.

The presence of symptoms is the means by which one recognizes that plants are affected with virus diseases, and the symptom-complex is utilized in distinguishing the different viruses. Virus diseases in plants produce chronic disorders that are usually systemic and that are characterized by any one or more of the following symptoms: chlorosis, necrosis, and stunting or overgrowth. Affected plants are seldom killed outright, but localized dead areas may form on any of the above-ground organs. Recovery from infection by a virus disease in plants is

unknown or at least uncommon. It has been reported to occur, however, in the case of sugar cane mosaic and ring spot of tobacco.

Ordinarily the development of symptoms of a virus disease is contingent upon the presence of young cells. Symptoms of ordinary tobacco mosaic do not appear, for example, in mature leaves of tobacco if the plant is inoculated, yet the sap from these mature leaves is capable of communicating the disorder when it is applied to the young leaves of another plant. All new growth on the first plant, however, which appears subsequent to inoculation, will be mosaic-affected.

Inclusion bodies have been found in mosaic-affected plants in the case of a sufficient number of virus diseases so that question of their possible etiological relation has been given special consideration. No accord has been reached, however, as to their exact nature. Some investigators regard these inclusions as the virus itself, others as products of the invaded cells, and others as the virus included in a mantle of altered cellular substance.

Within the past few years an increasing number of investigators are inclined to the idea that a virus represents filterable, invisible, non-cultivable states of bacteria. Transformation or transmutation of a bacterium to a virus or from a virus to a bacterium has, however, not been accomplished. Rivers (1932 a) is of the opinion that virus may be "situated along the line that separates inanimate incitants from minute living organisms." It would appear entirely probable that some viruses may be non-living, others living. Practically all investigators who have studied virus diseases during the past fifteen years are quite convinced that viruses are animate organisms. When it is recalled that some viruses approximate the size of the hemaglobin molecule (i. e., 5 to 8 millimicrons, the order of 10^{-6} mm.), the question may be justly raised, as was done by Rivers (1932 a), whether so small an aggregate of molecules could imaginably be capable of metabolic and reproductive functions. He concludes that "if viruses are minute, obligate parasites, in-

capable of multiplication in the absence of living susceptible host cells or if they are products of cellular perversion reproducible in series, it is useless to try to cultivate them."

Transmission of Viruses

If the seeds from a virus-affected plant are sown, the resulting seedlings are free from disease in nearly every instance. This is a most significant reason why virus diseases are not universally present on many kinds of plants. If ordinary tobacco mosaic virus were seed-borne, one would expect all tobacco plants the world over to be affected. Ring spot of tobacco and bean mosaic, however, are among the few disorders of virus origin in which the planting of infected seed may initiate the disease.

While, in general, virus diseases are highly infectious, as has been repeatedly demonstrated in the case of ordinary tobacco mosaic, some of them are difficult to communicate. Man himself is undoubtedly the primary vector for ordinary tobacco mosaic virus, and, in the writer's opinion, for many of the other highly infectious virus diseases. A body of evidence has been presented that such is the case with a mosaic disease of Narcissus and with rugose mosaic, spindle tuber disease, and leaf roll disease of potato. Various species of insects, of both biting and sucking types, mostly of the latter, transmit certain of the less easily communicable virus diseases, as typified by the sugar cane mosaic and the curly top of sugar beets. Most of the insects that are concerned with the dissemination of plant viruses belong to the *Homoptera*. There are included herein such groups as the leaf hoppers *(Jassidae)*, lantern flies *(Fulgoridae)*, whiteflies *(Aleyrodidae)*, plant lice *(Aphididae)*, and scale insects *(Coccidae)*, certain species of each group of which are known to be vectors.

Aphids are vectors of a larger number of virus diseases than any other group of the *Homoptera*. They are known to transmit potato leaf roll, mosaics of

such crops as crucifers, beans, celery, lettuce, and strawberries, onion dwarf, rosette of peanuts, and raspberry mosaic. Apparently, transmission of virus by insects is not purely mechanical in many cases, since it has been impossible to communicate certain diseases except by the appropriate insect. Aster yellows can be communicated only by *Cicadula sexnotata*, streak of maize only by *Cicadulina mbilia*, curly top of beet, in North America, only by *Eutettix tenellus*, and Kroepoek only by *Bemisea gossypiperda*. Another feature of some of the insect-transmitted viruses is that the insects do not possess the ability of transmitting the virus immediately after feeding upon a diseased plant. *Cicadula sexnotata*, for example, does not possess infective power until about ten days after having fed upon an aster affected with yellows, or upon some other species subject to aster yellows. This interval in the case of *Eutettix tenellus*, that transmits the virus of curly top of beets, is twenty-one to twenty-four hours.

In addition to this, there is evidence that certain species of grasshoppers *(Orthoptera)* and beetles *(Coleoptera)* transmit some of the potato mosaics, cucumber mosaic, and cowpea mosaic. Insects of another group, the thrips *(Thysanoptera)*, are capable of communicating such diseases as bean mosaic, sugar cane mosaic, and pineapple mosaic, spotted wilt of tomato, spinach blight, and streak of maize.

Classification of Viruses

Within recent years a body of evidence has been accumulated which shows that certain virus diseases are not an entity but are caused by two or more viruses. This appears to be true in the case of ordinary mosaic of tobacco, of ring spot of tobacco, of "breaking" in tulips, and of certain potato diseases. There are manifestly several distinct diseases in the case of certain crops, notably potatoes and tobacco, that are caused by distinct viruses. Eleven mosaic diseases of tobacco are recognized by James Johnson (1927) and eighteen by E. M. Johnson

(1930). In addition, several other mosaic diseases have been described on tobacco. Each of these virus diseases is distinguished, in part, on the basis of symptoms produced. Other differential or diagnostic features include differences in properties of the viruses, such as differences in longevity, cross-inoculability, tolerance to chemicals, thermal death points, effects of dilution on infectivity, and modes or agencies of transmission of the viruses. As a result of the studies which have brought to light these differences, there has come into being a body of methods of study, which, although not wholly satisfactory, are useful and serviceable in differentiating the several viruses. No uniform system nor standard of classification has been adopted, however, and in consequence this matter is yearly becoming more and more involved. There is a tendency with each kind of plant that is observed to be mosaic-affected, from the time of Mayer's (1886) report to the present, to ascribe it to a new virus. Confusion has arisen, furthermore, because the same virus has been found to react differently in different suscept species. Blodgett (1927) found, for example, that when Bliss Triumph potatoes are inoculated with ordinary tobacco mosaic virus, local necrotic lesions result, but there is no transmission to the succeeding crop. When, however, Green River potatoes are inoculated with the same virus, necrotic streaks appear in the stems and veins, and this streak disease is transmitted through the tubers to the succeeding crop. The disease is thus systemic in the one case and non-systemic in the other.

It has been shown also that certain species can serve as "carriers" without themselves exhibiting symptoms. This is true in the case of *Physalis alkekengi* and *Nicotiana glauca* that have been inoculated with ordinary tobacco mosaic virus. Again, cucumber mosaic can be transmitted from tobacco to tobacco by aphids, but these insects are incapable of transmitting ordinary mosaic from tobacco to tobacco (Hoggan, 1931). Smith (1934) has shown that separation of a mixture of potato x-virus and potato y-virus in the same potato plant can be accomplished

by the use of aphids. Tobacco plants inoculated with a mixture of the two viruses show irregular, necrotic lesions and severe necrosis of the veins. If the aphids have fed on an infected potato plant and are transferred to White Burley tobacco, dark green patches will eventually appear on the tobacco plant. These spots are the result of y-virus. If the affected potato leaf is pierced with a needle, and another White Burley plant is then inoculated with this needle, necrotic lesions and severe necrosis of the veins result. If then the necrotic tissues are punctured, and the needle is used to inoculate Jimson weed *(Datura stramonium)*, and after symptoms appear in the Jimson weed, the virus is passed back to tobacco, concentric rings appear on the tobacco. This is the result of the x-virus. Both viruses can again be brought back to tobacco, and the symptoms are like those first formed, namely, local necrosis of the veins, and the killing of intervein tissues.

Procedures looking toward standardization of classification or nomenclature of viruses, which admittedly are much needed, have been suggested by Johnson (1927) and by Johnson and Hoggan (1931). It appears to the writer, since viruses constitute a distinct group of pathogenic agents that have properties distinct from bacteria and fungi, that clarity and accuracy of expression of ideas concerning them would be enhanced by a distinctive terminology. The following proposals are therefore made to stimulate the attainment of this end:

1. Virus, the disease producing, pathogenic or pathogenizing agent or principle that causes disease (mosaics, ring spot, leaf curl, yellows, etc.).

2. Virusate, virusize, or enphytize, instead of inoculate, to transmit, communicate, transfer, or implant a virus.

3. Virosed, or virosized, instead of infected; virosive, instead of infectious.

4. Virusation, virusization, or enphytization, instead of inoculation.

5. Virosization, instead of infection. The phenomenon of infection, as applied to pathogenic bacteria and fungi, implies two ideas: (a) increase of inoculum by growth, and (b) response or

reaction of the suscept to the pathogen, thus resulting in symptoms. Viruses are not known to be animate or living; hence they may not be capable of growth. They may increase in the presence of living cells, i. e., *in vivo* but not *in vitro*. In place of being spoken of as animate or living things, since it is not known that they are alive, they might simply be regarded as biologic products, a generalized connotation.

The translation of the rather loose, inexact terminology used in much of the existing literature on diseases caused by viruses would probably constitute a handicap for some investigators, and has not been attempted in the discussions which follow.

It should be apparent to the reader, before he has reached this point, that no brief treatment of viruses and the diseases that they produce can give an adequate idea of this subject. Additional features will, of course, appear in the accounts of the several virus diseases of tobacco to be discussed in this same chapter. This also will be insufficient. The status of our knowledge of plant viruses, in general, is contained in a recent book (Smith, 1934), to which the reader is referred. It is all too apparent to the student of virus diseases that future progress in this problem will depend largely upon a better understanding of the nature of the viruses themselves. This understanding may result if light from the fields of chemistry and physics is brought to bear in a manner that will supplement the facts, regarding the nature of viruses, gained from the field of biology.

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Tobacco Mosaic

The publications dealing with tobacco mosaic are more voluminous than those concerned with any other tobacco disease. This may be attributed to two factors; namely, to the universal distribution of mosaic in all areas where tobacco is cultivated, and to the fact that tobacco mosaic is the first plant disease to be attributed to a virus as a causal agent.[1] The major proportion of these numerous investigations has been concerned primarily with the nature and properties of the causal agent, and the interpretation of results has led to widely different conclusions. A relatively small proportion of these investigations has dealt with the epiphytology of the disease and with its control, matters which have engaged the writer's attention for a period of years.

Mayer (1886) first demonstrated the infectious nature of mosaic to cultivated tobacco. No other species of plants were known to be subject to this disease until 1907, when Clinton (1907) showed that tobacco mosaic may be transmitted to tomato. Allard (1914 b) first conducted an extensive study of suscept relationships in the *Solanaceae*. He listed several species and varieties of *Nicotiana, Lycopersicum, Physalis, Datura, Petunia,* and *Capsicum* as suscepts. This list has been increased by various investigators, including Elmer (1925), whose account summarizes the status of this problem. These studies show that tobacco mosaic is not confined to the *Solanaceae*, but that fifteen interfamily and eleven interorder transmissions have been obtained. These include, in addition to those already mentioned, such cultivated suscepts as corn, sugar cane, bean, raspberry, cucumber, crook-neck squash, celery, and eggplant. This finding

[1] Recently Jones (Jones, L. R., The oldest known plant virus disease. Sci. n. s. **79**: 385. 1934) called attention to the fact that the so-called "breaking" of tulips is traceable through about four hundred years of recorded tulip culture. It is only recently, however, that "breaking" of tulips was found to be caused by a virus.

does not imply that mosaic diseases of these crops are identical with the ordinary tobacco mosaic, for some of them are known to have mosaics entirely distinct from the tobacco mosaic. Moreover, tobacco mosaic is now known to be caused by a complex of viruses, not by a single strain of virus.

Symptoms

Visible Changes.—Mosaic is attended by certain well-marked morphological changes in the leaves, stems, and floral organs. The roots do not appear to manifest any symptoms. A number of factors are concerned in the character of these changes which modify their intensity. These include age and vigor of the plants at time of inoculation, and such external factors as make up the soil environment and the atmosphere environment of the tobacco plant.

The most striking symptoms are summarized by Allard (1914 b) as follows: (1) partial or complete chlorosis, (2) curling of the leaves, (3) dwarfing and distortion of the leaves, (4) blistered or savoyed appearance of the leaves, (5) mottling of the foliage with different shades of green, (6) dwarfing of the entire plant, (7) dwarfing and distortion of the flowers, (8) blotched or bleached corollas (in *Nicotiana tabacum* only), (9) mosaic-affected sucker growth, and (10) necrosis or death of tissues—necrosis is sometimes very marked as in *Nicotiana rustica, N. acuminata, N. langsdorffii, N. sanderae, N. glutinosa,* and *N. alata.*

On young plants the first visible symptom is a slight downward curling and distortion of the innermost or bud leaves. They may be somewhat chlorotic. As these leaves unfold abnormally, dark green spots appear that develop rapidly into large, irregular, crumpled swellings or blisters. The lamina or blades of such leaves do not develop normally, so that the leaf may appear disproportionately long.

If plants are approaching maturity at time of inoculation no evidence of disease may appear on the older leaves. All new ones, however, and all suckers will develop

FIG. 44. Mosaic-affected leaf of tobacco. (From U. S. Dept. Agr.)

the mottle top or gray top form of mosaic with irregular patches of dark green and light green involving the entire leaf surface. Holmes (1931) has shown that yellow lesions, that remain localized, may form on older leaves if the inoculum is applied by pricking the leaves with pins.

Distinct symptoms appear in the flowers of pink-flowered varieties, although none of the greenish, purplish, yellow, or white-flowered ones exhibit symptoms (Allard, 1914 b). The corollas may be spotted and misshapen. Anthers, filaments, pistils, and capsules may contain the virus. Since germinable seed always gave healthy offspring (Allard, 1915 b), Allard believed the embryos were not invaded. It is possible that when the embryo is infected it is killed.

Usually malignant symptoms appear in *Nicotiana rustica,* resulting in the death of the leaf tissues. Such is the case, moreover, in Burley tobacco at the time when the leaves are approaching maturity. Cook (1931) noted that small, dark, necrotic spots may appear overnight in both the dark green and the light green areas on tobacco in Puerto Rico.

Histological Changes.—As might be anticipated, there are striking anatomical differences between normal and healthy leaves. Woods (1900) appears to be the first to point out that the palisade parenchyma of the lighted colored areas is not well developed. He seems to have been of the opinion that the dark green areas are normal and healthy. He figured the cells of the palisade parenchyma as four to five times as long as broad, whereas in moderately diseased areas these two dimensions are about the same. The transition from abnormal to normal areas is very abrupt. Dickson (1922) says that this occupies a lineal space of only three to four slightly affected cells. Chapman (1917) confirmed Woods's anatomical findings except that the parenchyma cells are more closely packed in the dark green areas, which he also regards as diseased, than in normal ones. Furthermore, the palisade in the dark green areas is always well

developed. Dickson (1922) made an exhaustive study of this phase of the problem and summarized his observations as follows:

(1) Hypoplasia of the lighter green areas manifest especially in the palisade; in consequence, such areas are over one-third less in thickness than darker green areas.

(2) Reduction of intercellular space volume in lighter green areas.

(3) Reduction of chlorophyll content because of fewer chloroplasts, less chlorophyll per plastid, or breaking down of plastids.

(4) Presence in the cells of secondary contents.

(5) Small hyaline bodies apparently derived from fragmented plastids occur in diseased chlorenchyma.

(6) Reduction in area covered by each epidermal cell over hypoplastic chlorenchyma.

(7) Transition from hypoplastic to non-hypoplastic tissue occupies in lineal space only 3 to 4 slightly affected cells.

(8) Most hypoplastic tissues may die, hence the occurrence of brown flecks.

(9) Darker green areas are more or less hypertrophied with more intercellular space and greater chlorophyll content than normal, and such areas are covered with epidermal cells that are larger in area than normal.

Cytological Changes.—The cytological changes that are associated with mosaic have been extensively investigated, and the results of these investigations are quite in accord. The interpretation of these findings and their significance, however, are quite diverse.

All investigators are agreed on the existence of one or more types of cell inclusions in mosaic tissues. One of these types, which from its appearance is designated "striate material," has been described by Goldstein (1924, 1926), Hoggan (1927), and Johnson and Rawlins (1925). The striate material is often in contact with the nucleus, and Rawlins and Johnson regard it as either the product of the diseased cell or as the causal agent. Since the virus is known to occur in cells in which no

striate materials exist; namely, in mature cells in which there are no apparent cytological derangements, it seems improbable that they can be regarded as the cause of mosaic.

In addition to striate material, large, spherical, vacuolate bodies are present. Likhité (1929) states that these bodies divide by constriction, and that they can be seen to conjugate in pairs. Goldstein (1924) also noted vacuolate bodies which she regarded as having the powers of autonomous movement independent of circulation by protoplasmic streaming. This observation was not confirmed by the findings of Smith (1926). Palm (1922) had earlier mentioned the presence in mosaic tissues of small granules with a maximum diameter of 0.5 μ. Dickson (1922) found very small, deeply straining bodies 0.3 μ in length, probably identical with those seen by Palm (1922) and with those described by Dufrénoy (1928) as mitochondria.

Dufrénoy (1928, 1929 a, 1929 b) and his associates concluded that the chloroplasts in the pale green diseased tissues undergo fatty degeneration, and that their stromata collapse. In the dark green tissues the cells first show an excessive metabolic activity. As the disease progresses, the protein crystals in the cells increase in size and in numbers. They hold that the vacuolate bodies are invisible without staining. By applying Nile blue, as a vital stain, the bodies became blue, and with osmic acid, brown, thus indicating their lipid nature. The striated bodies reacted like albuminoids with Denigès reagent, erythrocin, or syrupy phosphoric acid.

Physiological and Biochemical Changes.—Two important changes take place within mosaic tissues, one of which involves the disturbed metabolic activity of the cells and the other of which results in what has been interpreted as a quantitative increase in the infective principle itself.

In the first category, mention should be made of the disturbed enzyme relationships. Chapman (1917) found that the catalase activity of diseased tissues was below

that of normal ones of the same age, and furthermore that while the activity in the dark green portions of mosaic leaves approaches that of healthy leaves, in the light green areas it is much less than normal. He also found that diseased leaves contain more oxidase and peroxidase than normal ones. Dunlap (1931) found that starch was more readily converted into simpler compounds in mosaic than in healthy plants. This suggests that there is an increased diastic activity in mosaic-affected tissues. Holmes (1931), however, by staining with iodine found much less diastatic activity within the cells of local lesions. These discrepancies may, perhaps, be accounted for by difference in age of the tissues and lesions.

FIG. 45. Leaves malformed, in mosaic.

Dunlap (1928) and Peterson (1931) found that the quantity of the two pigments chlorophyll a and chlorophyll b is reduced in mosaic-affected tissues. The latter found, in addition, that there were less carotene and xanthophyll in affected cells.

As might be anticipated, the mosaic disease is accompanied by a disturbed carbohydrate content. In seedlings that were inoculated fifteen days prior to the date of analysis, Dunlap (1931) noted an increase in all carbohydrates except reducing sugars. His data agree with those of other investigators in showing that in mosaic leaves there is a reduction in starch, dextrins, pentosans, disaccharides, and reducing sugars.

The average hydrogen ion concentration of the expressed sap of mosaic leaves was found by Harvey (1920) to be pH 5.245 as compared with pH 5.380 of healthy leaves, showing that the active acidity of mosaic-affected leaves is somewhat greater than that of healthy ones.

From computations on the basis of green weight, Silberschmidt (1930) reported finding, in comparative analyses of the nicotine content of mosaic and healthy leaves, that affected leaves contained more nicotine than normal ones.

The most remarkable reaction concerned in mosaic tissues is the spread of the virus from cell to cell until it reaches all portions of the plant excepting the seed. The virus occurs throughout all portions of the roots, stem, and leaves. This spread within the plant is accomplished by the apparent increase in quantity of the virus itself, as shown by studies on its rate of spread from the point of inoculation. Allard (1914 b) determined, by inoculating the tips of leaves of a series of plants and then removing certain of the inoculated leaves at definite intervals after inoculation, that a period of approximately three days is required for the virus to spread from the tip of an average-sized leaf to the stalk. Grainger (1933) determined that the mosaic virus moves and "multiplies" at a logarithmic rate that may be portrayed by an S-curve. A graph of this type, as is known, represents

the typical growth curve of living organisms, and also the rate of accumulation of the catalytic agent in an autocatalytic reaction. Purdy (1928) has been able to secure evidence of the multiplication of virus in detached leaves by utilizing the fact that it spreads from the point of inoculation. White (1934) maintained it in excised tomato root tips.

The path of migration of the virus is believed to be along the protoplasmic strands or plasmodesms that extend from cell to cell. Caldwell (1934) maintains that it moves in all directions from the point of inoculation and independently from the food materials. Modification of environmental conditions has been shown to result in a masking of symptoms of mosaic and the attenuation of the virus. Johnson (1926) found that the symptoms are partly or wholly masked when the mosaic plants are kept at a temperature of 35° to 37° C. for ten days or more. New leaves developed at these temperatures show no symptoms of mosaic (Johnson, 1921). The virus from such plants, moreover, when used to inoculate other plants, gave rise to rather milder symptoms than when the inoculum was obtained from plants grown at temperatures of 25° to 30° C.

The recovery from mosaic symptoms has also been reported to result from variations in illumination of affected plants. Lodewijks (1910) concluded from experimentation that suffused light checked tobacco mosaic, red light made the symptoms less intense, and blue light cured mosaic. Chapman (1916, 1917) kept mosaic plants under blue cloth-hoods and secured a depression of macroscopic symptoms. Orange hoods caused an intensification of symptoms; red hoods, a diminution of differences in color between normal and mosaic-affected leaves. All affected plants when exposed for a short period to normal daylight exhibited symptoms in undiminished intensity. His most significant result was that there was no change in the virulence of the virus, regardless of the character of the light to which the affected plants were exposed. This fact was determined by using the sap from

plants exposed to the different lights to inoculate plants grown in daylight. Dickson (1922) subsequently confirmed the results secured by Chapman.

Arthur and Newell (1929) treated mosaic plants with ultraviolet light and found that it inactivated the virus and was lethal to the tissues of the tobacco plants as well.

The Disease

Names

The disease of the tobacco plant to which the name "mosaic" is properly applied is one of the most widespread maladies of this crop. It is known locally within the United States by a variety of names including "calico," "mottle top," "gray top," "foxy tobacco," "brindle tobacco," "mongrel tobacco," "chlorosis," and "walloon." In the flue-cured-tobacco belt this last name is of common usage among growers. The name *walloon,* applied to the Belgian-French, may have been originally employed as a term of derision. In some tobacco-growing sections the name "water-pied" is most commonly employed; in others, especially in Kentucky and Tennessee, the name "dry weather frenching" is used for this disease and "wet weather frenching" for a disorder to which the name "frenching" is applied elsewhere. In Germany it is designated *Mosaikkrankheit* or *Fleckenkrankheit;* in Netherlands, *bunt, fäule, mozaiekziekte, witkop,* or *vlekziekte;* in Hungary, *mozaikbetezege;* in Brazil, *molesta de mosaico;* in Italy, *male del bolla;* in France, *la mosaique vrai, la nielle vrai, mauche, rouille blanche,* or *maladies des taches blanches;* in Java, *bontong* or *ginje;* in China, *peh sem;* and in southern Russia the Tartars call it *bosuch.*

The basis of our present understanding of tobacco mosaic was laid by Mayer (1886) in 1886. Some writers maintain that it was first mentioned by van Swieten in 1857, but the writer finds nothing in van Swieten's report (1857) which indicates that he was dealing with tobacco mosaic. Fukushi (1929), in his résumé of tobacco mosaic, maintains that the disease has been known in

Japan since 1857. Mosaic probably occurs in all countries where tobacco is grown. It has been reported from all of the tobacco-growing areas within the United States and also from such other parts of the world as Germany, France, Italy, Netherlands, Russia, the East Indies, South Africa, Australia, the West Indies, Argentina, and Brazil.

Importance

Mosaic does not result in the death of cultivated tobacco plants, but dwarfs them and so modifies the leaves as seriously to affect both the quality and quantity of the crop. The two factors that have most to do with mosaic damage are the proportion of the plants which are affected, since this may vary from a few plants only to the entire crop, and the age of the plants when infection occurs. In general, the younger the plant at time of inoculation, the greater the injury. Clinton (1915) pointed out that mosaic deleteriously affected the burning quality and aroma, so that buyers discriminate against a mosaic-affected crop by an unfavorable price. Some growers, therefore, pull up and discard diseased plants rather than risk this reduction in price.

Estimates of losses from mosaic are hard to obtain because there are too few available data on reduction in yield and impairment in grade as are believed to result from this disorder. Chapman (1917) estimated that 13 per cent of the crop in the Connecticut Valley was mosaic, and that the losses that year over the entire United States were in excess of a million dollars. Valleau and Johnson (1927 a) estimated the losses in the Burley tobacco section as 3 per cent in 1927. Their observations from experimental plots show a reduction in market value of 60 per cent when Burley tobacco was inoculated at time of setting, and about 25 per cent when inoculated at topping time.

McMurtrey (1929) found that inoculation of Maryland tobacco at time of transplanting reduced the yield 30 to 35 per cent and the gross value of the crop 50 per cent. When the plants were inoculated a month after

transplanting, the damage from mosaic was practically as great. If inoculation was delayed until the crop had reached the topping stage, there was no reduction in yield, but the quality of the cured tobacco was impaired. Similar results from experimental plots have been recorded by Wolf and Moss (1933), who found that mosaic decreased the value of flue-cured tobacco 50 per cent, 42 per cent, and 23 per cent, when the plants were inoculated at time of setting, a month later, and at topping time, respectively. Losses comparable with the latter one are of common occurrence in the flue-cured-tobacco districts, where mosaic may be prevalent on essentially all plants after the crop has been topped.

Cause

The voluminous literature that has resulted from the numerous investigations that have sought to determine the cause of tobacco mosaic show that it is due either (1) to a disturbance of enzymotic equilibrium within the plants, (2) to abnormal environmental conditions, (3) to bacteria, (4) to protozoa, or (5) to a specific virus. At present it is generally accepted that mosaic is due to a virus or to a group of viruses.

Enzymes.—That mosaic is due to an enzyme or to enzymes as a primary factor was first maintained by Woods (1902) in the United States and by Heintzel (1900), working independently, in Germany. Woods found that there was an increased activity of oxidizing enzymes in diseased cells, as has been verified subsequently by several investigators. He states that enzymes prevent the proper elaboration of food materials, and as a result, the nutrition of the cells of growing points and of rapidly dividing cells is deficient. When on decay of the roots, stems, or leaves of affected plants, this enzyme is liberated into the soil, it may again be absorbed by the roots of seedlings transplanted into this soil, and thus the disease continues to exist from year to year. Freiberg's (1917) studies of the reactions of the infective principle and those of Vinson and Petre (1929) show that it is

analogous in many ways to a chemical substance. Freiberg noted that the infective principle is adsorbed by talc, a characteristic of all colloidal compounds including enzymes. The specificity of reaction of the infectious principle of mosaic and the unbalanced carbohydrate relations between lighter green and darker green areas, combined with the contention that formaldehyde is one of the first products of photosynthesis, were the bases upon which Freiberg rested his "mosaic enzymes" idea. He found, as had Allard (1917), that concentrated solutions of ether, chloroform, carbon tetrachloride, toluene, acetone, or glycerine do not destroy the infectious principle. Such temperatures, moreover, as inactivate most enzymes destroy its infectious properties. Mayer (1886) first noted that a temperature of 80° C. destroyed the mosaic agent. When mosaic juice is precipitated with aqueous safranin, the infectious material is inactivated and can be released by means of amyl alcohol (Vinson and Petre, 1929). These same investigators salted out the virus with ammonium sulphate and magnesium sulphate, protein precipitants. Allard (1916 b) has shown that if hydrogen peroxide is added to mosaic sap to destroy the peroxidase, the sap is still infectious. If 70 to 80 per cent alcohol is added, the peroxidase is precipitated, and the infectious principle is destroyed. The extracted sap will, on standing, lose its peroxidase content, but still remains infectious, and if treated with varying strengths of formaldehyde for thirty-one days, in a concentration of 1:1500, is not infectious, while in a 1:1200 concentration it gave infection, and both gave intense reactions for peroxidase. Concentrated peroxidase and catalase, moreover, from healthy plants are not infectious. These studies by Allard may be interpreted as very convincing in demonstrating that neither enzymes nor other normal constituents of healthy plants can be considered responsible for mosaic.

Environmental Factors.—The studies on environmental factors as causes of mosaic are for the most part scattered fragmentary statements of observation or opin-

ion. Woods (1902) believed that injury to the roots, when the seedlings are transplanted, produced mosaic. He said there is evidence that rapid growth caused by too much nitrogen or too high temperature is favorable to the disease, and that this is connected with the manufacture of reserve nitrogen and its distribution to the rapidly growing parts. He noted, as did Sturgis (1898), that close clayey soils are not favorable for a uniform growth of leaves, since, on such soils, if extremes of soil moisture content exist, plants respond by too rapid or too slow growth, and in consequence mosaic develops. When such soils are loosened by cultivation or are given partial shade, the incidence of mosaic is decreased. Protection afforded by cheese-cloth covers is said to diminish the amount of mosaic.

All attempts to prove that mosaic is due to a lack of specific mineral nutrients have given concordant results in showing that it is not to be regarded as a deficiency disease, although various workers state that certain minerals influence the extent of mosaic. Koning (1899), for example, says that kainit and Thomas slag diminish its prevalence. Hunger (1902, 1905) thought that unfavorable conditions for growth led to the production of specific toxins within the tobacco plant that ultimately resulted in mosaic. He was under the impression that the disease was in part due to nutritional deficiencies, and that injury to the roots in transplanting predisposes the plants to mosaic. Even though he (1908) later found that mosaic is infectious and could be communicated by touching first the leaf of a mosaic, then of a healthy plant, he maintained the belief that it was of non-parasitic origin.

Lodewijks (1910) thought mosaic could be cured by colored light, as has previously been stated, which Chapman (1917) and Dickson (1922) proved was due merely to a masking of symptoms, since the sap from these "cured" leaves was still infectious.

Bacteria.—Mayer (1886) appears to be the first adherent to the bacterial nature of the mosaic disease. He arrived at this conclusion not by cultures and inocula-

tions but by failure to find animal or fungous parasites in mosaic-affected plants. All his efforts to inoculate with such bacteria as he isolated failed to produce mosaic. Nevertheless, he thought that the specific pathogen must reside in the soil in which affected plants were growing, and in consequence recommended a rotation of seed beds and of fields devoted to tobacco culture. Iwanowski (1903) described the occurrence in mosaic tissue of bacteria-like bodies. Among the recent workers who employed the advances which have been made in our knowledge of culturing bacteria to the mosaic problem are Bonequet (1917) and Dickson (1922). The former mentions a nitrate-reducing *Streptococcus* in mosaic tissues, but did not prove its relation to the disease by subjecting it to Koch's postulates. The latter surface-disinfected mosaic leaves, made tissues plantings in bouillon, and used these bouillon cultures to inoculate healthy plants. He secured infection; but in the light of Allard's (1914 b) finding that a dilution of 1:10,000 of mosaic sap is still infectious, one may regard the bacteria as secondary forms. In summary of the bacterial view, it may be said that it is wholly unsustained at present.

Protozoa.—Iwanowski (1903) first directed attention to the amoeba-like bodies in the cells of mosaic plants. Little consideration appears to have been given this view until after the appearance of a brief cytological paper by Palm (1922) in his study of mosaic in Sumatra. He observed foreign corpuscles in intimate contact with or in the vicinity of the nucleus in mosaic, but not in healthy tissue. Those bodies are amoebiform, spherical, and vacuolate or reticulate. They vary in size from 0.5 - 1.5 μ but possess no power of locomotion. Palm concluded from this work that tobacco mosaic belongs etiologically to the chlamydozoanoses, and designated the cause *Strongyloplasma Iwanowskii*. The more recent studies by Hoggan (1927), Rawlins and Johnson (1925), Goldstein (1924, 1926), Smith (1926), Duggar and Armstrong (1923), and others have led to the conclusion that there

occur in mosaic cells rounded to amoeboid "X-bodies." These are not constituents of healthy tissues. They have no autonomous movement, and are not related directly as the cause of mosaic, but more probably as an effect. Likhité (1929), however, thinks that these bodies represent a living organism for which he proposed the name *Vacuolarium iwanowski* n.g., n.sp.

Recently Jones (1926) described a slime mold, which he designated *Plasmodiophora tabaci* n.sp., occurring in mosaic tobacco plants. In criticism of this work, it may be said that he did not eliminate the possible introduction of the infective principle with this organism in his inoculations, and therefore one cannot safely draw conclusions from his tests of pathogenicity.

Virus.—The virus theory of causation of mosaic has gained most general acceptance. Allard's numerous papers (1914 a, 1914 b, 1915 a, 1915 b, 1916 a, 1916 b, 1918) have done most to lead to this conclusion. He summarizes his studies by stating that, since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe it is an ultramicroscopic parasite of some kind. Beijerinck (1899) was among the first to express this general idea of the nature of the infective agent. Since he found that the filtrate from mosaic sap that had passed through a Chamberland filter was infectious, he assumed that the agent is a non-corpuscular, fluid-like material that had the power of growing when in contact with meristematic cells—a living, contagious fluid or as he called it a "contagium vivum fluidum." By means of filtration experiments, Duggar and Karrer (1921) found that this infective agent is particulate, and that the mosaic particles pass filters with dimensions permitting hemoglobin to pass. From their determination the sizes of mosaic particles were found to be about 30 m μ, or about of the proportion 30:1000 when compared with bacterial plant pathogens, and with a volume relation of 1:37,000 if one regards mosaic particles as spheres.

Properties of the Infective Principle

The properties of the infective agent of tobacco mosaic may be classified as (a) biological, (b) chemical, and (c) physical.

Biological Properties.—Under this caption are included (1) the infectiousness or capacity of the virus to produce mosaic and (2) the capacity of the virus to increase quantitatively. It has long been known that mosaic results when the sap of mosaic-affected tobacco plants is communicated to healthy ones. Symptoms of mosaic will appear eight to fourteen days after inoculation on young plants. This interval may be as short as four to five days if young leaves are inoculated by piercing them with a contaminated pin. If mature leaves are inoculated, ordinarily no symptoms will be evident in them, but the sap from such inoculated mature leaves becomes infectious; furthermore, all new leaves formed subsequent to inoculation will be mosaic-affected on plants to whose mature leaves the inoculum was applied. All terminal growth and lateral suckers on such plants will be mosaic affected.

Quantitative increase of the virus rests upon evidence like that secured by McKinney and Webb (1926). They used a definite quantity of mosaic sap to inoculate a healthy plant and then after the disease had developed used another definite quantity of sap from the infected plant to inoculate a second plant. They repeated these sub-inoculations into plants with the development of symptoms in each one of the series until the dilution of the inoculum had reached a point corresponding with one part in ten to the thirty-third power. Allard (1915 a) found that when mosaic sap is diluted with water beyond 1 part in 10,000 the virus particles become so widely dispersed that infection is not likely to result from inoculation with the diluted sap.

If mosaic sap is diluted with the sap of healthy tobacco plants or with that from other species of plants, it may lose its infectivity if the mixtures are allowed to stand for a few days. Duggar and Armstrong (1925)

found that one part of mosaic sap when diluted with five parts of poke weed sap may completely lose its infectivity. McKinney (1927) found that the sap from cucumber or Jimson weed decreased the infectivity of mosaic tobacco sap proportional to the relative concentration of the foreign sap to the mosaic sap. Whether this loss of infectiousness is due to adsorption or to some chemical interaction of the saps is not known.

One of the peculiarities of the tobacco mosaic virus, a property that it possesses in common with all viruses, is its inability to increase in artificial culture media; that is to say, it is not known to increase except in the presence of living cells. The claim was made by Olitsky (1925) that mosaic virus is cultivable. His culture medium was prepared by mincing 80 grams of tomato plant tissue, mixing it with 250 cc. of sterile water, then centrifuging for 1 hour at 1500 - 2000 revolutions per minute. The supernatant liquid was then passed through two Berkefeld N filters, tubed in 3 to 5 cc. portions, and held for seven days at 28° to 30° C. to insure sterility. The inoculum consisted of 0.1 to 0.2 cc. of sap drawn with capillary pipettes from diseased stems. Dilutions were made in series using 0.1 to 0.2 cc. from the first culture. Plants were inoculated with each subculture. Olitsky claimed that infection resulted up to the twelfth subculture, which represents a dilution of 4×10^{-16}. Mulvania (1925) and Goldsworthy (1926) were unable to confirm Olitsky's work and secured no infections after the third dilution or subculture. Grainger (1930) also failed to get an increase of virus in culture media made by extracting dried tobacco leaves or in culture on chloroplasts prepared according to a special technique. Johnson's investigations[2] constitute the most thoroughgoing attempts that have yet been made to cultivate the ordinary tobacco mosaic virus. He used as a medium the expressed sap of healthy tobacco plants, sterilized by filtration. He also chemically fractionated the sap from healthy plants and used the va-

[2] Johnson, F. H., A new approach to the problem of culturing *in vitro* the infective principle of tobacco mosaic, 1932. Unpublished thesis, Duke University.

rious fractions as media, after they had been suspended or dissolved in sterile distilled water. In no case was there any evidence of increase of the virus in these media. Recently White (1934), by means of tissue cultures of excised roots, has been successful in culturing the virus.

Chemical Properties.—Precipitation of the virus, together with certain other constituents of expressed sap of mosaic plants, occurs upon the addition of such reagents as are ordinarily used in the precipitation of proteins. In studies on the purification of the virus, Vinson (1927, 1929) showed that if mosaic sap is centrifuged at 2000 revolutions per minute to remove the larger particles, and if the supernatant liquid is cooled to 0° C., a precipitation of virus occurs upon the addition of 2 volumes of acetone at -8° C. If then the precipitate is collected on filter paper and washed with ether or absolute alcohol at -8° C., and the ether or alcohol is allowed to evaporate, the precipitate may then be dissolved in water, and is highly infectious. The filtrate, however, is non-infectious. Barton-Wright and McBain (1933) isolated a crystalline white compound from mosaic sap that contained no nitrogen but was highly infectious, which indicates that the virus is not a living principle but a chemical compound. Caldwell (1934) claims, however, that the crystals are those of KH_2PO_4 to which the virus adheres as an impurity.

The recent studies by Stanley (1934) dealing with the effects of the proteolytic enzyme, pepsin, indicate that the tobacco-mosaic virus is protein in nature. He was able to inactivate the virus by digestion of infectious sap stored at favorable temperatures.

Ammonium sulphate or magnesium sulphate, both protein precipitants, "salt out" the virus along with the proteins, and this precipitate retains its infectiousness.

Allard (1917) found that concentrated solutions of ether, chloroform, carbon tetrachloride, toluene, acetone, or glycerine do not destroy the active principle, as has previously been stated. He also (1918) used various acids, alkalis, and germicides in the same manner. Nitric

or hydrochloric acid was harmful to the virus when the concentrations approximated one gram of acid to 50 to 100 cc. of expressed sap. Phosphoric, citric, or acetic acid destroyed infectivity when employed in concentra-

FIG. 46. A stubble that remained alive throughout the winter in North Carolina and produced, in the spring, a mosaic-affected sucker.

tions approaching one gram in 20 to 50 cc. of expressed sap. The infectivity of the virus was modified but little by aluminum sulphate, magnesium sulphate, sodium chloride, mercuric chloride, sodium nitrate, lead nitrate, silver nitrate, or lithium nitrate. Compounds of phenol in concentrations lethal to bacteria exerted but little effect upon the virus. Chloral hydrate, napthalene, camphor, or thymol had no effect upon infectiousness of the virus.

Physical Properties.—One of the best-known physical properties of the tobacco-mosaic virus was first demonstrated by Beijerinck (1899). He found, as have scores of other students of mosaic, that the sap of affected plants, when passed through filters of such a character as to remove all bacteria from the filtrate, is still capable of producing the disease. Duggar and his associates (1921, 1924), from filtration experiments, concluded that the diameter of the virus particles must approximate as a maximum 30 to 35 millimicrons. Waugh and Vinson (1932), however, state that their experiments with purified virus indicate that the particle size is of less diameter than 10 millimicrons. Smith (1934) determined by filtration that the particle size is 15 to 20 millimicrons.

Grainger (1933) inoculated tomato plants with virus from mosaic tobacco and found that the water naturally exuded by guttation from the infected tomato leaves was non-infectious to tobacco. Duggar (1929) found that mosaic sap that has been ground for twelve hours with diatomaceous earth is still infectious, whereas bacteria, including *Bacillus subtilis*, were destroyed in much less time when subjected to similar treatment.

Inactivation of the virus can be accomplished by a hydrostatic pressure of 130,000 pounds or more (Giddings, Allard, and Hite, 1929).

The virus may be adsorbed by kaolin, or aluminum hydroxide gel, as shown by Fukushi (1933). Adsorption, he found, was increased if the reaction of the fluid in which the kaolin was suspended was less than pH 6.0. By the addition of dilute ammonia the virus was eluated,

and then if the eluate were rendered slightly acid, virulence was regained.

The influence of aging on the infectivity of tobacco mosaic virus depends upon several factors. Dry mosaic leaves, kept in a closed jar in semi-darkness, were found to be infectious after thirty-one years (Valleau, 1927 c). The sap of mosaic plants, kept in a closed flask and protected from bacterial decomposition by a layer of toluene, may remain infectious for five years (Dickson, 1925). If, on the other hand, the sap is allowed to stand at room temperature, exposed to fermentation, it loses its ability to produce infection in a short time (Mulvania, 1926, and Holmes, 1928). Allard (1916 b) has shown that infective sap frozen in liquid air to -180° C. for fifteen minutes loses none of its infectiousness.

The virus of mosaic in a dry state is less susceptible to inactivation than in a wet state. Dried mosaic leaves will retain their infectiousness after being heated for a half hour at 120° C., but may lose it if exposed for an equal period at 140° C. (Allard, 1916 b). Sap that is air dried and then heated to 100° C. for a half hour is no longer infectious. Fresh sap was reported by Mayer (1886) to be non-infectious when heated to 80° C. for five minutes. Mulvania (1926) found that sap could be heated as long as twenty days at 80° C. without losing its ability to cause tobacco mosaic. The temperatures required to inactivate fresh sap appear from several investigations to be between 88° and 90° C.

Concerning the action of light on tobacco-mosaic virus, there are some wide discrepancies in experimental results. This is probably due in part to failure to eliminate temperature effects, desiccatory effects, differences in light intensity, and quality of the radiations which were able to reach the virus particles. This matter has been studied among others by Mulvania (1926), by Arthur and Newell (1929), and by Duggar and Hollaender (1934 a, 1934 b). The last-named investigators found that the virus of ordinary tobacco mosaic is much more tolerant to light than are such bacteria as *Bacillus sub-*

tilis, B. megetherium and *Serratia marcescens,* the resistance ratio of virus to bacteria being about 200:1. They used a fresh suspension of semipurified virus and exposed it to wave lengths in the range of 2537 to 6120 A. The materials were exposed, in quartz cells, to a capillary mercury vapor lamp, and the temperature was maintained at 1° to 2° C. by means of ice. They found that inactivation occurs at wave lengths shorter than 3100 A. The greatest influence was exerted at 2652 A, at which wave length one-hundredth the energy was required to produce a perceptible effect as at 3100 A.

Immunity and Resistance

Whether any species or varieties of *Nicotiana* are resistant to ordinary mosaic remains doubtful. Apparrently the only instance of resistance in tobacco is that reported by Nolla, Guggenheim, and Roque (1934), in the case of a variety called *Ambalema.* This variety was first observed in Colombia to be free from symptoms. It may merely be a symptomless "carrier," as is known to be the case with *Nicotiana glauca.* The recent studies by Kunkel (1934) on acquired immunity, and Beale (1934) on serological reactions, contain a summary of the present status of these matters. Kunkel (1934) found that *Nicotiana sylvestris* may be immunized against aucuba mosaic of tomato by inoculation with tobacco-mosaic virus. A lapse of about two days is required between inoculation and complete immunization, and during this period there may be no visible change. If the tobacco leaves are inoculated directly, the immunized areas correspond closely with the areas inoculated. If the infection is systemic, then a period longer than two days is required for the plants to become immune. Leaves too old to exhibit visible symptoms acquire immunity very slowly, sometimes requiring four to six weeks. The virus of aucuba mosaic does not multiply appreciably within immunized cells.

Similarly he was able to demonstrate that tobacco plants inoculated with attenuated strains of tobacco-

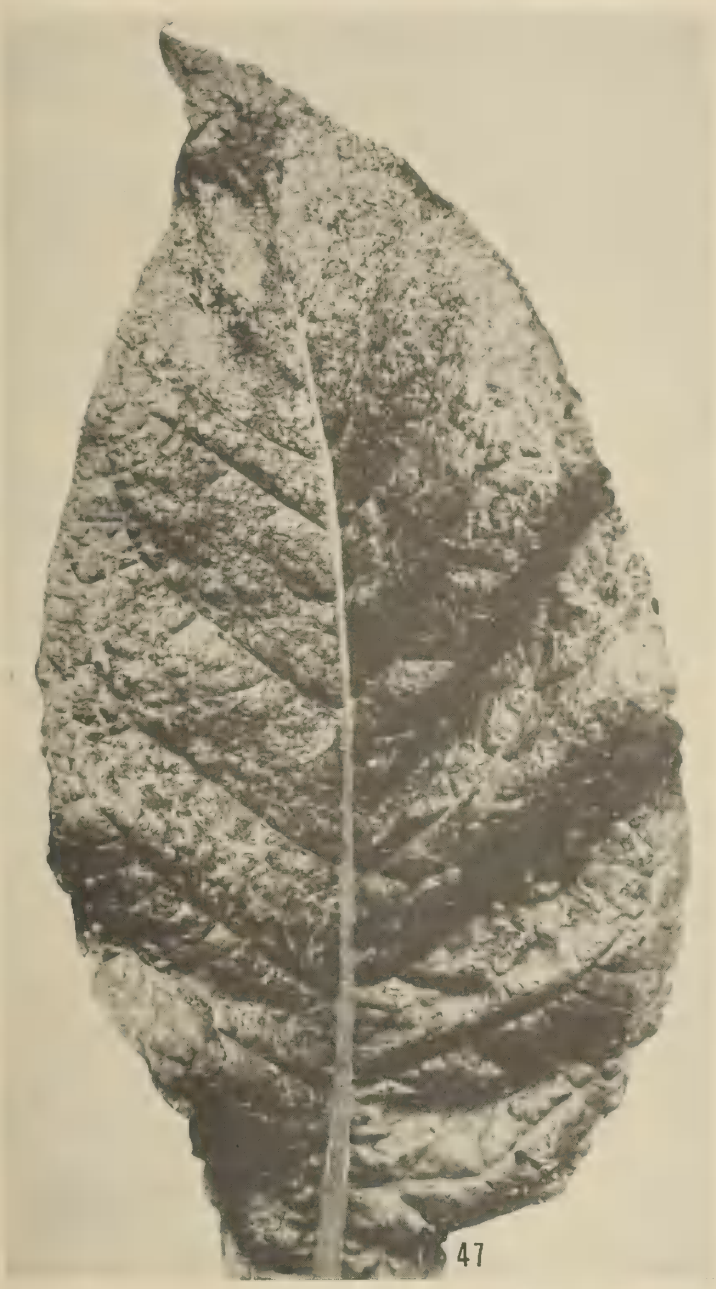

FIG. 47. Mosaic may cause the death of tissues, in which case numerous brown spots appear.

mosaic virus became immune to tobacco mosaic. On the other hand, cucumber-mosaic virus and the virus of ring spot, when used as inoculum on tobacco, afforded no protection against aucuba mosaic.

Thung (1931) found in tobacco plants inoculated with virus of ordinary tobacco mosaic that the symptoms of ordinary tobacco mosaic fail to develop if the plants are infected with white mosaic (Johnson's virus 6) or vice versa.

Valleau[3] does not interpret these results as evidences of immunity, but that when cells are parasitized to the limit by one virus, it is impossible to parasitize them with another virus.

The evidence from the several investigations on virus diseases of plants shows that antisera can be produced, and that precipitins form in the presence of the homologous antigen. This whole matter and its relation to the specific identification of plant viruses are carefully considered in the investigations of Beale (1934).

Epiphytology

The writer's studies on the epiphytology of tobacco mosaic in the flue-cured district of North Carolina extend over four successive seasons, and had for their purpose the determination of (1) how the tobacco mosaic virus passes the winter, (2) how it is spread in the seed bed and in the field, and (3) how cultural practices influence its incidence and prevalence. The procedure was essentially that which would be employed by an epidemiologist in studying any particular disease that becomes epidemic among humans. No effort was made to interfere with or to modify current farm practices. A record of the crop grown on all fields was kept as a means of determining the possible freedom of the soil from infectious material or of its possible contamination with such material as tops, suckers, stubbles, and secondary sucker growth, that might reasonably be expected to serve

[3] From a paper read before the American Phytopathological Society, December 27, 1934.

as a source of mosaic virus for a succeeding crop. Each seed bed was examined one or more times and each selected field four or five times, at intervals of ten to fifteen days, from the time that the tobacco was transplanted to the beginning of harvest.

Sources of Mosaic in Seed Beds.—Early in the course of these studies it was noted that mosaic occurs on seedlings of sufficient size for transplanting. Such factors as slope, exposure, rainfall, location of the seed beds, and variety of tobacco were noted to be without modifying influence on the occurrence of mosaic among the 584 beds studied. Of these, 108 contained mosaic seedlings. The possible agencies by which mosaic virus might be expected to be carried over the winter and transmitted to the seedlings include the following: (1) by survival of the infectious principle in tobacco refuse or trash in the soil; (2) by survival of the virus on canvas or seed-bed cloth and on frames; (3) by use of contaminated or infected seed; (4) by use of tobacco refuse spread over the beds as a repellent against flea beetles (*Epitrix parvula* Fab.); (5) by use of manure in which tobacco trash had been incorporated as a fertilizer material; (6) by overwintering of the virus in solanaceous weeds; (7) by transmission of the virus by insects; and (8) by the grower himself, through the careless use of manufactured tobacco.

Although Johnson and Ogden (1929) found that the virus can retain its infectivity for long periods of time in Wisconsin, in dead plant material incorporated with the soil, and on contaminated seed-bed cloth, these are not important means of introducing mosaic virus into seed beds in the flue-cured areas. Only one of the 108 infected beds examined was located on the site of an old bed.

Seed saved from mosaic-affected plants produced, in six trials, healthy seedlings. That mosaic is not seed-borne was earlier concluded from observations by Chapman (1917), Olitsky (1925), and from critical experiments by Allard (1915 b), who injected virus into placental tis-

sue of young capsules with the result that such seed as developed and were germinable gave healthy offspring.

Tobacco refuse was used as a repellent against flea beetles in 2 of the 108 infected seed beds that the writer examined. Mosaic virus may have been introduced with the trash in these cases because the virus in 1 among 6 lots of flue-cured mosaic leaves was determined to have retained its infectiousness. When tobacco refuse is used as a source of mineral nutrients for commercial fertilizers, there is little likelihood of its being a source of virus for the reason that tobacco stems are processed before being incorporated with the mixed fertilizer. It is inadvisable, of course, to use stems that have not been processed or sterilized. Even if fresh mosaic-affected leaves are mixed with the soil when the seed are sown, mosaic may not appear on the seedlings, as found by the experiments of Allard (1914 b) and of the writer. Raciborski (1900) and Moutia (1928) placed inoculum consisting of fresh mosaic leaves under the roots, and a portion of the plants only became mosaic.

Although such solanaceous weeds as horse nettle *(Solanum carolinense)* and black nightshade *(Solanum nigrum)* are of common occurrence in seed beds in the flue-cured district, observations indicate that they are of little significance in the epiphytology of tobacco mosaic. They may be of importance in other tobacco-growing regions, and every effort should be made to eradicate them from the vicinity of the beds.

Flea beetles, although commonly present in seed beds, do not appear to be vectors of mosaic virus, as indicated by the lack of any apparent correlation between their presence and the presence or relative abundance of mosaic-affected seedlings.

Aphids were not found in any diseased seed beds, and were noted in only 6 of the 584 beds that were examined. Hoggan (1929) found that the peach aphid (*Myzus persicae* Sulz.) transmits cucumber-mosaic virus from tobacco to tobacco, but is not able, under identical conditions, to transmit tobacco-mosaic virus. Three other aphid

species, however, *M. pseudosolani* Theob., *M. circumflexus* Buckt., and *Macrosiphum solanifolii* Ashm., were shown by her (1931) to transmit the cucumber-mosaic virus readily from both tomato and tobacco. These three species appear unable to transmit the tobacco-mosaic virus from tobacco to tobacco, but can transmit it from tomato to tobacco. There was evidence also, under the conditions which she used, of the occasional transmission of the tobacco-mosaic virus from black nightshade to tobacco by *Myzus pseudosolani.*

It was early suspected, as the result of repeated observations, that the tobacco used by the grower is a source of inoculum in seed beds. He could effect inoculation while working in the seed beds by spitting on plants or by handling them after the hands had become contaminated through contact with the mouth. Direct evidence of the infectivity of manufactured tobacco has, in fact, been presented by Valleau and Johnson (1927 c), Fukushi (1931), and Busch and Wolf (1933). Confirmatory evidence, which is wholly epiphytological, rests upon the following observations, which, if considered as a unit, show that manufactured tobacco may be infectious:

1. Seed beds are free from mosaic at first, and the disease may be found in some of them about two weeks after the beds have been weeded, when only an occasional plant may be affected.

2. Mosaic is first apparent in other beds ten to fourteen days after the most mature plants have been removed in order to set them in the field.

3. Mosaic is not found in other beds until near the close of the planting season. Observations made each season on the course of development of the disease support these three conclusions. In 1931, for example, 65 seed beds were seen at three successive intervals, first about twelve days prior to transplanting, again during the first days of transplanting, and a third time about twelve days later. One bed contained mosaic seedlings at the first examination, 5 at the second, and 11 at the third.

4. In seed beds in which the disease occurs early in the season, the number of infected plants increases with each successive examination, when the interval between observations is a period of ten to fourteen days.

5. Mosaic-affected plants always occur singly or in groups composed of two or three plants standing near each other. If infested soil was the source of inoculum, groups of a considerable number of affected plants should be found instead of occasional plants standing singly. Then, too, there was no evidence, except in 1 of the 584 beds, that the virus could have remained dormant in the soil, since all except this one were apparently situated on non-infested soil. Delayed infection in infested soil, as shown by Johnson and Ogden (1929) to occur in Wisconsin, does not appear to be a factor in the flue-cured districts. If healthy plants, set with aseptic precautions in places from which mosaic plants had been removed, become infected, such infection is evident as soon as the new leaves appear, an interval of eleven to eighteen days being ample.

6. The oldest leaves of diseased seedlings appear normal; only the bud leaves exhibit symptoms. It has long been known that mature leaves, when inoculated, never appear mosaic, although the sap from such leaves is infectious; whereas young leaves, when inoculated, are distinctly mottled ten to fourteen days later.

These observations lead to the conclusion that the virus was introduced by the grower, and that manufactured forms of tobacco constituted the most probable source of the introduction of mosaic virus into 105 of the 108 seed beds examined in the course of the writer's studies.

Sources of Mosaic in Fields.—It is evident that the presence of mosaic in seed beds might be responsible to a significant extent for mosaic in the fields. Clinton (1915) concluded that infected seedlings are probably primarily responsible for most of the mosaic in fields in Connecticut. Chapman (1917) stated that primary infection in Massachusetts can usually be traced to seed beds. Even though only occasional plants are diseased when

drawn from the beds, a large proportion become inoculated as a result of handling in the operations of pulling and setting.

The occurrence of mosaic in seed beds could not, however, be made to account for all cases of mosaic in fields. It was noted early in the writer's epiphytological studies and verified repeatedly each season, that mosaic not only appears on seedlings while they are still in the seed beds, but that it may appear in the field when there was no evidence of mosaic in the beds from which these plants were drawn, provided a diseased crop had been grown on these fields during the preceding year. The only exceptions to this were occasional fields in which mosaic was introduced late in the development of the crop from a near-by infected crop. This observation suggested contamination of the worker's hands and survival of the virus in the field, as sources. The former was excluded because affected plants stood singly, and not in series, as would occur if they were set with contaminated hands.

Proof of overwintering in the field rests upon the following evidence: Tobacco plants remain alive, in North Carolina, throughout the winter and produce new suckers in the spring. Such suckers arise from portions of the stem beneath the soil. In many cases they are mosaic, though some are healthy. Furthermore, when the stubbles remain undisturbed from the close of harvest until the following spring, a large proportion of the roots remain alive. The virus might be expected to survive in these living roots, even though all parts of the stem were dead. In fact, as regards the writer's observations, when tobacco is planted on fields that have grown a mosaic-affected crop during the preceding year, it invariably becomes infected. There have been no exceptions in the 188 fields in which a detailed record has been kept or in other fields in which observations on this point were made, except in the few fields to be mentioned later, in which an attempt was made to eradicate mosaic by fall plowing.

When a field in which a cereal crop has been grown during the preceding year and another in which a mosaic-affected-tobacco crop has been grown are set with tobacco plants from the same mosaic-free bed, the former may be expected to be free of mosaic from the beginning and may remain so throughout the season, whereas the latter will contain occasional diseased plants that may be noted as soon as the seedlings have become established. Exceptions to this were noted in a total of 17 fields in which disease-free seedlings were set on newly cleared land or on fields that had not been cropped with tobacco for a term of years, yet these fields contained scattered mosaic-affected plants. The source of inoculum in these cases could not be definitely determined, but it appears probable that in resetting the grower himself inoculated them. He may have used manufactured tobacco which served as the source of inoculum through the careless contact of hands, lips, and seedlings, in turn.

Evidence of the survival of mosaic in the field is contained, furthermore, in the report of Johnson and Ogden (1929). They found that some of the plants set in April in infested soil were affected with mosaic about a month later. The virus in such cases had overwintered in the soil.

The occurrence of mosaic in the growing crop of flue-cured tobacco may be accounted for in the following ways: (1) by planting diseased or contaminated seedlings, (2) by planting disease-free seedlings on infested soil, and (3) by introduction subsequent to establishment of the seedlings in the field.

The writer employed the following criteria in order to determine the source of mosaic in fields. Seed beds alone were regarded as the source of inoculum for all fields that were set with mosaic plants, when such fields had not been cropped with tobacco for more than one year previously. Infested soils alone were regarded as the source when the seed beds from which the crop was planted were mosaic-free, and when such fields had borne a mosaic-affected crop during the previous year. When

there were mosaic plants in the seed beds and when such plants were set in fields that had borne an affected crop during the preceding season, both infected plants and infested soil were regarded as sources of inoculum. When disease-free plants were set on newly cleared land or on fields that had not been cropped with tobacco for a term of years, the sources of inoculum could not be determined. In such cases it was probably introduced by the grower from adjoining fields or from the careless use of chewing tobacco or snuff.

On the foregoing basis, 7 among 63 fields selected for repeated examination in 1928 became infested from the use of diseased seedlings, 27 from overwintering of the virus in the field, 22 from both infected plants and infested soil, and 7 from sources not definitely determined. Of 90 fields studied in 1929, 10 became infested from the use of diseased seedlings, 50 from overwintering of virus in the field, 25 from both diseased plants and infested soil, and 5 from sources not definitely determined. Of 76 fields in 1930, mosaic seedlings were responsible for the disease in 7, 48 became infested from overwintering of virus in the soil, 16 from both infested soil and mosaic seedlings, and 5 from sources not definitely known. Of a total, therefore, of 229 fields examined during the three seasons, the source of inoculum could be traced to seed beds alone in the case of 24, to infested soil of fields alone in 125, and to both diseased seedlings and infested soil in 63. In the case of 17 the source of inoculum could not be definitely determined.

Dissemination of Mosaic in the Field.—In considering the spread of mosaic in the field, attention was given to the following factors: (1) influence of the source of the initial infection; that is, whether the field was set with plants from a seed bed containing mosaic seedlings or whether the field itself was infested from a previous crop, or whether both of these sources contributed to the initial infection; (2) rate of spread as determined by examination of each selected field at intervals of ten to fifteen days and by recording the number of mosaic

plants in each row at successive dates of examination; and (3) agencies by which the virus was spread as determined by meteorological conditions, by cultural operations practiced in the interval between successive dates of examination, and by peculiarities in the course of dissemination during these intervals. Table 9 contains a summary of each season's observations on the number of mosaic plants at the beginning of the growing season, at the beginning of harvest, and at two intervening times.

TABLE 9

SUMMARY OF THE RATE OF SPREAD OF MOSAIC IN FIELDS OF FLUE-CURED TOBACCO

Year	Number of fields examined	Total number of mosaic plants at successive periods of examination				Total number of plants in the fields
		1st period	2nd period	3rd period	4th period	
1928....	63	3,488	8,962	20,112	29,212	123,048
1929.....	90	3,945	9,028	17,452	26,277	146,355
1930.....	76	4,464	9,607	23,402	36,477	144,505
Total..	229	11,897	27,597	60,966	91,966	413,908

Of the total of 413,908 plants examined during the three seasons, an average of 2.9 per cent was mosaic when first examined, 6.7 per cent at the next examination, 14.7 per cent at the next, and 22.2 per cent at the beginning of harvest. The extremes in these fields in percentage of infection at the date of initial examination varied from none to approximately 65 per cent.

It may be noted, first of all, that the percentage of mosaic increases progressively each season throughout the growing period. There is no sudden onset of mosaic following rains with attendant conditions favoring rapid growth of the crop, as many growers believe. The rapid spread which follows topping and which is not shown in this table is the result of wholesale inoculation consequent on handling each plant during topping. Prior to that time the crop is cultivated at least once each week. While hoeing, cultivating, worming, suckering, or priming off

the seed-bed leaves, workmen inadvertently spread the virus. Observations indicate that it may be communicated by any means, such as single-trees, traces, plow handles, garments, or hands, which effect a contact of diseased and healthy plants in turn.

Influence of Source of Inoculum on Spread.—It might be anticipated that a relationship should exist between the spread of mosaic and the source of inoculum. If the seed beds contain mosaic plants and such plants are set in clean fields, a larger proportion of the plants should be mosaic than if infested soils are set with healthy plants. Furthermore, the largest proportion of mosaic should occur in consequence of transplanting seedlings from infected beds into infested fields. Four representative fields among those studied in each of the three seasons have been selected in presenting data on the source of virus as a factor in the dissemination of the disease. The entire body of data on all fields would have been too cumbersome. The data assembled in Table 10 deal with 12 fields selected from the group of 24 fields, in which, it may be recalled, mosaic was brought to the field by means of diseased seedlings.

Table 10

Spread of Mosaic in Fields in Which the Disease Was Introduced from Seed Beds Alone

Year	Field Number	Percentage of mosaic plants on successive dates of examination				Total number of plants in field
		1st examination	2nd examination	3rd examination	4th examination	
1928	1	June 25, 7.3	July 9, 38.6	July 18, 75.3	.....	1,452
1928	2	June 16, 2.2	June 30, 7.4	July 12, 49.2	.....	2,880
1928	3	June 20, 2.1	July 2, 7.8	July 14, 24.3	.....	4,604
1928	4	June 9, 7.8	June 20, 29.7	July 6, 49.7	July 17, 75.6	964
1929	5	June 5, .4	June 17, 1.1	June 29, 6.9	July 9, 22.8	1,409
1929	6	June 5, 1.5	June 17, 5.2	June 29, 15.5	July 9, 41.0	3,400
1929	7	June 14, 14.8	June 28, 18.8	July 8, 28.9	.....	960
1929	8	June 3, 8.9	June 13, 11.7	June 28, 64.6	July 8, 71.6	1,552
1930	9	June 7, 32.5	June 20, 51.7	July 2, 65.8	July 12, 84.5	1,077
1930	10	June 6, 62.3	June 18, 78.8	June 28, 91.7	July 8, 98.3	1,806
1930	11	June 12, 23.9	June 23, 29.7	July 6, 46.6	.....	1,560
1930	12	May 31, 5.3	June 12, 37.1	June 23, 48.2	July 7, 85.1	2,194
Total	.....	.....	.....	.....	.....	23,858

It is evident, first of all, that there is no uniformity in percentage of infection in different fields. This is due to the fact that some seed beds contain only a few mosaic plants, while others contain many, and that some growers discard infected plants in advance of pulling plants, whereas others make no effort to discard or to avoid them.

In these 12 fields containing 23,858 plants, 2,648 or 11.1 per cent were mosaic at the date of first examination, 5,080 or 21.3 per cent at the second examination, 10,175 or 42.6 per cent at the third examination, and 12,740 or 53.4 per cent at the time when the first leaves were primed. In some fields the crops were mature at the third examination, and it was assumed that the same number were mosaic at the fourth period as were found at the third.

The distribution of mosaic plants in recently set fields is a criterion by which one can judge whether mosaic was present in the seed beds from which these fields were planted. Rows containing series of mosaic plants, from a few in consecutive order to practically entire rows, indicate that the disease was present in the seed bed. When mosaic was found in beds at the beginning of the season for transplanting, the fields set from these beds always contained series of diseased plants. When the beds were not examined until after it had been found that the fields contained series of mosaic plants, such beds were always found to contain mosaic seedlings. Moreover, mosaic plants standing singly indicate, as a rule, that the virus has overwintered in the field at the site where the new plant was set. One very striking exception has come to the writer's attention. This field had grown a crop of wheat during the previous year. A planting machine of the type in which each alternate plant is set by one of the two operators was used in transplanting. All plants set by one operator came from a mosaic-free seed bed, and all set by the other came from a bed containing mosaic plants. When this field was first examined, practically every alternate plant in 8 short rows was mosaic.

By actual count, 359 out of a total of 744 were affected. Cases similar to this have been noted previously by several investigators.

Twelve fields were selected among 125 studied in which the virus overwintered in the field and in which infested soil was the sole source of inoculum. The data on 12 representative fields are assembled in Table 11.

TABLE 11

DISSEMINATION OF MOSAIC IN REPRESENTATIVE FIELDS IN WHICH THE VIRUS OVERWINTERED IN REFUSE (STUBBLES, TOPS, AND SUCKERS)

Year	Field Number	Percentage of mosaic plants on successive dates of examination				Total number of plants in field
		1st examination	2nd examination	3rd examination	4th examination	
1928	1	June 12, 0.5	July 3, 5.4	July 16, 22.9	July 27, 29.9	956
1928	2	June 21, .2	June 30, .95	July 12, 3.0	July 23, 9.4	2,100
1928	3	June 19, 2.1	July 2, 9.9	July 12, 15.4	July 23, 32.5	1,712
1928	4	June 19, .2	July 2, .5	July 12, 1.1	July 24, 2.7	2,298
1929	5	June 8, .3	June 21, .6	July 2, 1.5	July 12, 3.0	1,800
1929	6	June 12, .9	June 25, 2.6	July 5, 5.0	July 16, 6.4	2,000
1929	7	June 12, 1.6	June 25, 4.9	July 5, 9.7	July 19, 11.1	1,360
1929	8	June 12, .46	June 25, 3.2	July 5, 10.1	July 16, 15.6	1,745
1930	9	June 10, .33	June 21, 1.6	July 1, 2.4	July 11, 3.4	1,200
1930	10	June 6, .31	June 20, .7	July 2, 10.0	July 19, 13.0	2,000
1930	11	June 7, 1.1	June 23, 3.0	July 7, 3.6	July 24, 22.9	2,033
1930	12	June 7, .3	June 20, 2.5	July 2, 7.7	.....	1,620
Total	.....	.....	.....	.....	.....	20,824

In these 12 typical fields containing a total of 20,824 plants, 143 or slightly less than 0.7 per cent were affected when first examined; 589 or 2.8 per cent, when next examined; 1,570 or 7.5 per cent, when next examined; and at harvest time 2,834 or 13.6 per cent were mosaic. There is strikingly less mosaic throughout the season when clean plants are set in infested soils than when diseased plants are set in clean fields, as may be seen by comparing the results in Tables 10 and 11.

Twelve representative fields were selected from the 63 fields studies in which both diseased seedlings and infested soils served as sources of inoculum. The data on these fields, comparable to those in Tables 10 and 11, are summarized in Table 12.

TABLE 12

SPREAD OF MOSAIC IN SELECTED FIELDS IN WHICH THE CROP WAS MOSAIC DURING THE PRECEDING YEAR AND WHICH WERE SET WITH PLANTS FROM INFECTED BEDS

Year	Field Number	Percentage of mosaic plants on successive dates of examination				Total number of plants in field
		1st examination	2nd examination	3rd examination	4th examination	
1930.....	1	June 7, 4.5	June 20, 11.4	July 2, 31.6	July 12, 55.8	1,888
1930.....	2	June 6, 15.9	June 18, 21.9	June 28, 64.7	July 8, 78.3	1,224
1930.....	3	June 7, 3.1	June 20, 5.8	July 2, 31.2	July 12, 37.7	2,000
1930.....	4	June 6, 36.5	June 18, 45.4	June 28, 75.0	July 8, 83.1	1,272
1929.....	5	May 30, 6.8	June 12, 16.2	June 25, 29.9	July 5, 52.4	1,835
1929.....	6	June 5, 3.8	June 17, 11.5	June 29, 19.8	July 9, 36.5	2,464
1929.....	7	June 5, 15.9	June 17, 30.2	June 29, 52.2	July 9, 76.3	778
1929.....	8	June 3, 12.9	June 13, 20.7	June 28, 34.8	July 8, 56.7	1,080
1928.....	9	June 19, 7.2	July 2, 23.5	July 14, 34.0	July 25, 37.1	1,170
1928.....	10	June 13, 23.2	June 25, 36.1	July 9, 58.8	July 18, 73.8	1,007
1928.....	11	June 9, 5.5	June 28, 31.5	July 12, 69.3	July 23, 88.7	597
1928.....	12	June 21, 20.8	June 30, 36.1	July 12, 62.2	July 23, 71.3	821
Total..	.....	.....	.....	.....	.....	16,136

These fields contained a total of 16,136 plants, of which 1,810 or 11.2 per cent were mosaic at the beginning of the growing season; 3,362 or 20.8 per cent, at the second examination; 6,703 or 41.5 per cent, at the third examination; and 9,376 or 58 per cent were affected at harvest time. On the basis of the data contained in Tables 10 and 11, one would have expected 11.7 per cent of the plants to be affected at the time of the first examination and 67 per cent at the beginning of harvest. When one takes into account the small number of fields considered in each tabulation and that 12 were selected at random among a total of 63 of the same type, these discrepancies are without significance.

RATE OF SPREAD OF MOSAIC IN THE FIELD.—It is evident from the data assembled in Tables 10, 11, and 12 that the proportion of plants that are affected at the beginning of the growing season, in any given field, is related to the source of inoculum. The subsequent rate of spread, however, is governed by a number of factors and is not necessarily determined by the initial proportion of healthy to mosaic-affected plants. One might reasonably ex-

pect the virus to be spread early over a large part of the field if there were many mosaic plants in the field early in the season, and that a much smaller proportion would eventually become diseased if there were few mosaic plants at first. This is not necessarily the case, as may be noted by comparison of any two of the fields among those summarized in Tables 10, 11, and 12. In order to give an adequate idea of the rate of spread, 6 fields were selected, some with a small proportion of plants that were mosaic as soon as the seedlings had become established after transplanting, and others with a large proportion of mosaic plants. The percentage of affected plants in 8 adjoining rows in these 6 fields is shown in Figure 48.

There is no apparent proportionality between the rates of spread in any of these fields. For example, field 1 has an initial infection of approximately 38 per cent and field 4 of 4.5 per cent, and at the beginning of harvest,

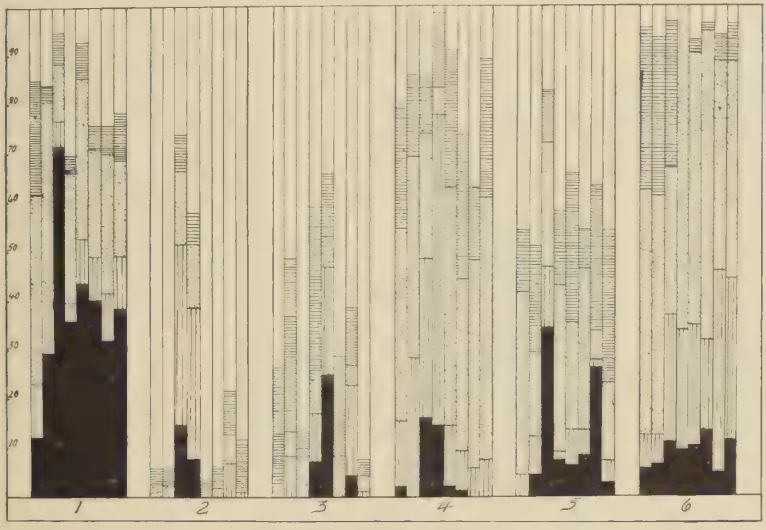

FIG. 48. Dissemination of mosaic in 8 adjacent rows in each of 6 representative fields. The portion in black represents the percentage of infection about two weeks after the seedlings had been transplanted. The portion shaded with vertical lines indicates the increase during the next ten to fifteen days. The stippled part represents the additional spread until the beginning of harvest. The unshaded portion represents the percentage that remained mosaic free at the beginning of harvest.

each is approximately 82 per cent mosaic. Field 3 has the same percentage initial infection as field 4, but by harvest time it is only 48 per cent mosaic. The percentage of mosaic at the beginning of harvest is greatest in field 6, being 96 per cent, whereas approximately 8.5 per cent of the plants were affected on the date of the first examination.

It may be noted from examination of Figure 48 that the same lack of correlation as obtains between fields in rate of spread obtains also between adjacent rows in any one of the fields. It may be seen, furthermore, that although a considerable number of plants in row 3 of field 2 were affected at first, no mosaic was found in row 5 until after the plants had been topped, and that none was found in row 2 until the third examination. Selby (1904) recorded a variation of 0 to 56 per cent of disease in rows on the Ohio Agricultural Experiment Station farm. In general, not only these fields, but all of the 229 fields, showed a "row-ward" spread of the disease. In explanation of this, it should be pointed out that all cultivation until the period when the crop has reached the stage for topping involves single rows. At topping, the grower usually tops two rows at a time, and consequently pairs of rows tend to have the same number of mosaic plants about two weeks afterward.

Agencies of Spread of Mosaic.—Hornworms *(Protoparce sexta)* have been shown by Elmer (1925) to be capable of transmitting the virus of mosaic. They do not travel from plant to plant and are, therefore, not a significant factor in the spread of mosaic. If flea beetles were responsible for the dissemination of the virus, the course of spread would tend to be radial in all directions from infected plants. If rain and wind were agencies of dissemination, the disease should spread in the direction in which the prevailing winds blow or in the direction of wind-blown rain. All field observations indicate that the disease is spread with the row. Plants nearest diseased ones and in the same row tend to be the first to contract mosaic. Fields have been noted in which a

certain row contained many mosaic plants at first and in which the disease continued to spread, while the rows on either side remained free from mosaic until near the close of the growing season. All the evidence in hand indicates that man is the primary agent of dissemination in the field through any cultural operation in which he effects a contact of diseased and healthy plants. These operations include the handling of plants while worming, suckering, or topping, and the brushing against diseased and healthy plants in turn while passing along the rows in hoeing and cultivating.

Koning (1899) conducted extensive field experiments and found that in ordinary topping operations 88 per cent of the crop may become inoculated. Van Bijlert (1899) concluded that the wholesale spread of mosaic in the Dutch East Indies was due to carriage of the virus on the hands of coolies during the operations of topping and suckering. When coolies searched every alternate plant for bud worms, beginning with a mosaic plant, and left half of the plants untouched, practically every plant which the workmen touched became affected, and the others, with few exceptions, remained healthy. Similar results were obtained from experiments in Sumatra by Hunger (1908). An experimental field in which the coolies were not permitted to work and which was separated from a badly mosaic field by a path 1 meter wide remained mosaic-free (Hunger, 1903). Clinton (1915) observed that mosaic is almost certain to develop on all healthy plants that have been touched by hands that have come in contact with the sap from mosaic-affected plants.

The writer noted in one field of approximately 4 acres, planted after wheat, that 3 acres were mosaic-free at first, and the remaining acre at one side of the field contained many affected plants. The grower cultivated from the mosaic-free side toward the diseased side in order to avoid spreading mosaic. In consequence, when the crop was mature, the space between adjoining rows marked the line of separation between diseased and disease-free portions. In another field containing about 2

acres, planted after tobacco, three mosaic plants were found on June 5. Each mosaic plant was staked to enable the grower to avoid touching these plants. No additional plants were affected on July 10, at the beginning of harvest.

Dissemination during Harvest.—In the case of a few fields, periodic observations on the spread of mosaic were continued each season throughout the harvest period. During this period each plant is handled from three to six times or even more in removing suckers and in priming off leaves. In all cases mosaic continued to spread, so that practically all plants were affected when the final examination was made at the close of harvest. The extent of this dissemination from the beginning to the end of harvest is shown, in the case of 14 fields, in Figure 49.

The crops in fields 3, 8, and 9 were harvested by cutting, and the fields were examined finally after suckers had appeared from the stubbles. The crops in all other

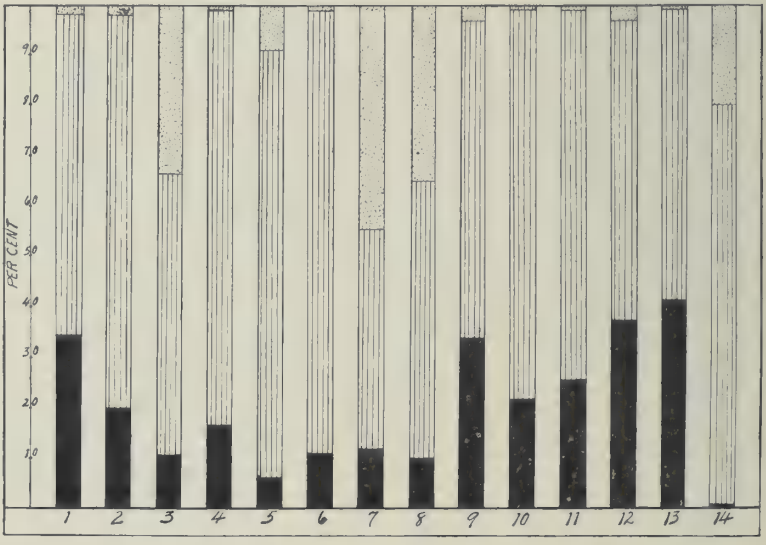

Fig. 49. Fourteen mosaic tobacco fields showing, in black, the percentage of each that was affected at the beginning of harvest; in the part shaded with vertical lines, the additional spread during harvest; and in the stippled areas, the percentage that was not mosaic by the end of harvest.

fields were primed. Mosaic is apparently equally prevalent whether the crop is cut or primed. These 14 fields contained a total of 21,561 plants, 3,865 or an average of 17.9 per cent of which were affected at the beginning of harvest, and 19,344 or 89.7 per cent of which were affected at the close of harvest. Fields 6 and 7 were essentially equally mosaic at the beginning of harvest, but the former was 99 per cent mosaic, and the latter was 55 per cent diseased at the end of harvest. Field 14, that was slightly less than 1 per cent mosaic when harvest was begun, was 80 per cent mosaic-affected at the close of harvest.

From the observations just described, it is apparent that the extent of soil infestation may be practically equal in fields that are seriously damaged by mosaic and in those without appreciable injury. By knowing the percentage of mosaic at the beginning of harvest, no one can foretell either the extent of initial infection in or the ultimate damage to a succeeding crop of tobacco on a given field when tobacco follows tobacco.

Influence of Continual Cropping with Tobacco on the Incidence of Mosaic.—Eleven fields were selected among the 229 examined in order to show the possible relationship between soil infestation and the incidence of mosaic. In these fields tobacco was grown for at least four consecutive seasons, and there was no apparent source of inoculum except infested soil from the previous crop. The relative percentage of mosaic and healthy plants at harvest each year in each field is shown in Figure 50.

Fields 1, 2, 3, 5, 7, 8, 9, and 11 had more mosaic in 1928 than in any other year. Fields 1, 3, 4, 5, 7, 9, and 10 had more in 1929 than in 1930. Field 5 alone had about the same proportion of mosaic each year. There does not appear to be any consistency in the proportion of healthy and mosaic plants in any two fields in any of the three years. For example, field 2 had 1,110 affected plants among a total of 1,790 in 1928, and field 4 had 40 among 2,230. The next year field 2 had 130, and field 4

had 239. In 1930 the corresponding numbers were 250 and 218, respectively. Neither is there any correlation between the amount of mosaic in one crop and that in another on the same field in consecutive years. Field 9, for example, had 790 diseased plants among 2,120 in 1928, 205 in 1929, and 50 in 1930.

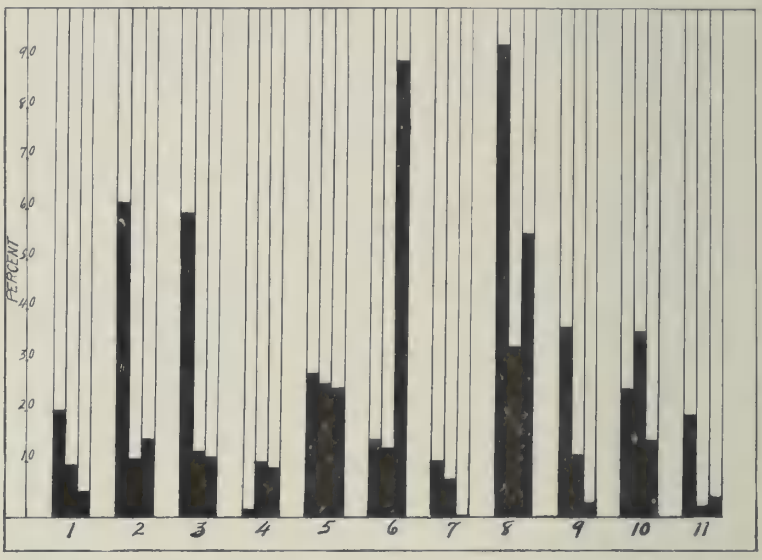

FIG. 50. Eleven fields, represented by triple columns, that were continuously cropped with tobacco in the seasons of 1928, 1929, and 1930. In each field the column at the left represents the field in 1928; the middle one, that of 1929; and the one at the right, that of 1930. Each column shows the percentage of mosaic and mosaic-free plants at the beginning of harvest, the shaded area indicates the mosaic-affected portion and the clear area, the mosaic-free portion.

INFLUENCE OF PLOWING OUT STUBBLES AFTER HARVEST.[4]

When it became apparent that the virus can overwinter in stubbles, an effort was made to learn whether plowing out diseased stubbles as soon as possible after harvest would be of benefit in decreasing mosaic in the succeeding year. The observations that follow bear on this point.

[4] Lehman (LEHMAN, S. G., Contaminated soil and cultural practices as related to the occurrence and spread of tobacco mosaic. N. C. AGR. EXP. STA. BUL. **46**: 43 pp. illus. 1934) has recently shown the beneficial effect of plowing under or of removing diseased plants in the fall.

A field in which practically all plants were affected in 1928, as the result of introduction from a diseased seed bed, was plowed in August of that year, and the upturned stubbles were gathered and removed from the field. It was planted with healthy seedlings in 1929, and that portion under observation, which contained 2,520 plants, remained free from disease until June 25, when the crop was ready to be topped. Mosaic was present in the adjacent portion from the time the plants had become established. Apparently mosaic had been introduced about ten days earlier from the adjacent diseased portion during the operation of worming. By harvest time 98 plants had become affected. In another field about one-quarter of the plants were mosaic in 1928 at the beginning of harvest. The stubbles were cut down shortly after harvest, and the field was then plowed. No mosaic was found in this field of 909 plants until June 21, when there were 2 diseased plants, and at the beginning of harvest it contained only 11 mosaic plants. In 1929, in a portion of a field with 1,170 plants, 434 were affected when the crop was ready to be harvested. This field was plowed and the refuse turned under during late August. In 1930 it was set with healthy plants, and 6 were found to be mosaic on June 12. By July 16 the total number of affected plants in this field was 79.

In 1929, 402 of the 1,404 plants in another field were mosaic. This field was plowed shortly after harvest, and the refuse was turned under. Only 4 plants in the succeeding crop on this field were affected.

At the Tobacco Experiment Station, Oxford, N. C., in 1929, all of the plants in 2 plots, totaling about 3,000, were mosaic as the result of inoculation at time of setting or about four weeks afterward. The affected stubbles were removed from these plots when they were plowed early in September. In 1930, 2 mosaic plants were found in this area when examination was made soon after the seedlings had established themselves after transplanting.

Three of these 5 fields, therefore, were freed from

soil infestation by plowing out the stubbles, as determined by the fact that no mosaic plants were found in them until the plants had reached the stage for topping. The evidence from these observations and from a considerable number of others, when taken together, shows that the plowing out of mosaic stubbles is beneficial. Its efficacy results from hastening the decay of stubbles and, in consequence, the destruction of the virus. It cannot be relied upon, however, as a means of entirely freeing fields from infestation during the period from September to the following May.

Influence of Fallowing for One Year.—Throughout the flue-cured-tobacco area, fields are sometimes left fallow as a means of increasing their productiveness. Such a practice could reasonably be expected to serve as a means of ridding fields of infectious material. Unfortunately for this study, of the 68 fields that were examined in 1928, only 4 were not cultivated in 1929, but were again cropped with tobacco in 1930. In one of these fields 286 among a total of 956 plants were mosaic in 1928, and only 3 of the 1,100 plants set on the same area in 1930 were affected. In the second field 424 of the 1,346 plants were mosaic in 1928, and of the 1,261 grown in the same field in 1930, 653 were mosaic. In the third field, 688 of the 821 plants were diseased in 1928, while of the 939 set in the same field in 1930, 79 were mosaic. In the fourth field, among a total of 2,940 plants, 205 were diseased in 1928, and on the same area in 1930 there were 95 mosaic-affected plants among 3,015. The seed bed from which the second field was set in 1930 was mosaic, which accounts for the large amount of disease in this case. In the other fields the few diseased plants, which stood singly at first, may have become mosaic from the survival of the virus in old stubbles or, as is more probable, from contaminated plants used in resetting. The only conclusion that appears warranted from these observations is that one year's fallowing is insufficient to free infested fields of mosaic.

Control

The investigations of this phase of the mosaic problem show that mosaic may be prevented from occurring and that its spread can be controlled, once it does appear.

The observance of certain sanitary measures is of primary importance in preventing its occurrence in the seed beds. Woods (1902), Allard (1917), and Valleau and Johnson (1927 b) stress sterilization of the seed-bed soil. This is imperative unless new seed-bed sites are employed. The avoidance of stems or trash as a fertilizer material for seed beds is suggested (Clinton, 1915, and Valleau and Johnson, 1927 b), since they are known to serve as a source of inoculum. Johnson and Ogden (1929) found that the virus retains its infectivity in old leaves in the soil.

Tobacco mosaic is not seed-borne, a point on which all investigations are in accord, so that seed treatment is ineffectual in preventing mosaic.

It is known that certain solanaceous weeds are carriers of mosaic virus, indicating the need for their destruction, especially when growing in the vicinity of the seed beds (Valleau and Johnson, 1927 b). The perennial-rooted horse nettle that occurs generally in the flue-cured district, may be infected and yet exhibit no external symptoms, or at most scarcely perceptible symptoms. Nishimura (1918) has shown that one of the ground cherries, *Physalis alkekengi*, when inoculated with sap of mosaic tobacco, fails to show symptoms; and yet its sap, when used to inoculate tobacco, produced mosaic in 80 per cent of the plants inoculated.

Evidence from the writer's epiphytological studies shows that the careless use of manufactured tobacco constitutes the most common source of inoculum for mosaic in the flue-cured areas. This corroborates the direct evidence of infectivity of manufactured tobacco secured by Valleau and Johnson (1927 c), who found 4 brands of plug tobacco, 7 of cigarettes, and 5 of granulated tobacco to be infectious. Fukushi (1930) found that most sam-

ples of pipe tobacco are infectious, and Busch and Wolf (1933) that 2 brands of plug tobacco and 2 of snuff result in mosaic, when used as inoculum. Growers, who use tobacco while "picking" or "weeding" the beds, or while drawing plants for transplanting, may inoculate the seedlings by means of spittle.

There are three sources of introduction of mosaic into fields: (1) infected seedlings, (2) hibernation in the previous crop, and (3) transmission by man from an affected crop in a near-by field. In the writer's studies, the disease was carried to 24 of the 229 fields observed solely by means of infected seedlings. In such cases, mosaic is seriously destructive.

Mosaic virus may retain its infectivity in tobacco stubbles, as shown by the fact that in the spring mosaic suckers may develop on stubbles that have remained alive. In 125 of the 229 fields observed, the virus overwintered in tobacco stubbles. Mosaic almost invariably develops in fields where a diseased crop of flue-cured tobacco has been grown during the preceding year. This shows that crop rotation must be practiced. In the rotation system, an interval of at least two years between crops of tobacco on the same field appears to be necessary. The plowing out of tobacco stubbles early after harvest tends to decrease the likelihood of survival in the fields.

The spread of mosaic in the field tends to be rowward. All the evidence indicates that the grower, while tending the crop, is the primary agent for the dissemination of mosaic. This dissemination can be largely avoided when growers realize that indiscriminate handling of plants while hoeing, topping, worming, or suckering results in spreading mosaic. Insects, wind, and wind-blown rain do not appear to be consequential vectors in the field.

Roguing or removal of mosaic plants at time of the first cultivation has been found to be very effective means of checking the spread of mosaic (Wolf, 1933). Roguing is recommended in fields whose soil is infested when such fields are set with mosaic-free seedlings.

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TOBACCO RING SPOT

Our knowledge of ring spot is resultant largely from investigations conducted in Virginia, whence it was first described in 1922.

SUSCEPTS.—Apparently all agronomic varieties of *Nicotiana tabacum* are susceptible to attack, with no evidence of varietal differences in resistance. It has been noted on Adcock, Burley, Warne, Maryland, Lizard Tail, Macedonian, Little Orinoco, Green's Wildfire Resistant, and Kentucky Yellow (Wingard, 1928). The following botanical varieties of *Nicotiana tabacum* became infected following artificial inoculation: *atropurpurea, auriculata, brasiliensis, calycina, calyciflora, cavala, colossea, gigan-*

tea, lacerata, latissima, macrophylla, microphylla, purpurea, sanguinea, and Turkish.

Among other species of *Nicotiana* listed by Wingard (1928) as suscepts are *Nicotiana acuminata, N. clevelandii, N. glutinosa, N. langsdorffii, N. longiflora, N. paniculata, N. plumbaginifolia, N. quadrivalvis, N. quadrivalvis* var. *multivalvis, N. repanda, N. rustica, N. sanderae, N. suaveolens, N. sylvestris, N. tomentosa,* and *N. trigonophylla.* As the result of his inoculation studies, he found, moreover, that the disease is not confined to tobacco, but occurs on thirty-seven other genera representing seventeen families of plants. Such closely related plants as Jimson weed *(Datura stramonium),* nightshade *(Solanum nigrum),* ground cherry *(Physalis angulata),* horse nettle *(Solanum carolinense),* petunia *(Petunia violacea),* and eggplant *(Solanum melongena)* were found to be susceptible. Among the crop plants which he lists as suscepts are watermelon *(Citrullus vulgaris),* cucumber *(Cucumis sativus),* pumpkin *(Cucurbita pepo),* lettuce *(Lactuca sativa),* lima bean *(Phaseolus lunatus),* kidney bean *(Phaseolus vulgaris),* cowpea *(Vigna sinensis),* beet *(Beta vulgaris),* sweet clover *(Melilotus alba),* and okra *(Hibiscus esculentus).* Such common weeds as ragweed *(Ambrosia artemisiifolia),* sunflower *(Helianthus annuus),* morning-glory *(Ipomoea purpurea),* poke weed *(Phytolacca decandra),* lambs' quarters *(Chenopodium album),* and stick weed *(Verbesina alternifolia)* have been shown to be subject to ring spot infection.

Symptoms.—Ring spot is essentially a foliage disease of tobacco, although on some suscept species it occurs also on stems and fruit. Infection on the leaf is characterized by the development of concentric rings and irregular wavy lines of streaks.

The first symptom develops within three days after inoculation and appears as a small translucent ring of necrotic tissue, a millimeter or less in diameter, surrounding an island of apparently normal tissue. Within three or four days these necrotic tissues will have be-

come bleached and dry, and a second ring will have formed outside the primary ring and will be separated from it by a zone of green tissue. As this process continues, other rings form surrounding those previously formed. The tissues near the center of the spot, usually extending at least to the first ring, gradually die. The shape of the diseased areas is influenced by their position with reference to the veins of the leaf. If the spots are distant from the veins, they are circular; if they involve veins, they are irregular; and if they are centered on the larger veins or midrib, they tend to extend along the veins and have a lobed irregular or zigzag margin.

Infection may become systemic within ten to fourteen days and involve all young leaves in turn as they appear, or may gradually fail to develop on the new leaves and suckers so that only a faint grayish mottling can be detected toward the end of the growing season. Such

FIG. 51. Tobacco ring spot in which the rings of dead tissue tend to involve the tissues between the veins.

FIG. 52. Tobacco ring spot showing concentric rings and wavy lines that tend to follow the veins. (From Wingard.)

leaves, in which the symptoms have become masked, appear thicker and more leathery than normal.

Infection of the stems is indicated by dark, sunken lesions which may extend the entire length of the main stem. This symptom-complex has not been observed on commercial tobacco but on several other species, resulting finally in the blackening of the entire stem and eventual death of the plants (Wingard, 1928). Valleau (1932) distinguishes two distinct strains of ring spot virus in Kentucky which he calls green ring spot and yellow ring spot. The former produces the well-known chlorotic green patterns and the latter yellow ones, while the whole leaf turns yellowish green to nearly white. Both may occur on seedlings. Symptoms of the green ring spot may not appear on seedlings unless low temperatures obtain, but the disease does appear to check their growth in the seed bed; yellow ring spot-affected seedlings grow slowly, however, and are scarcely suitable for transplanting.

Ring spot-affected plants produce relatively few seed which appears to result from involvement of the pollen.

History and Importance of the Disease.—Ring spot was first definitely recognized in Virginia as a distinct disease by Fromme and Wingard (1922). It had doubtless been present for a period of years prior to their first notice of it in 1917. Subsequently it has been noted in Kentucky, Tennessee, Ohio, and North Carolina. It has been reported also from Russia, Sumatra, Australia, South Africa, and Nyasaland.

Ring spot has been found to be of common occurrence throughout Virginia. The incidence of infection of fields may be as high as 80 per cent or as low as 30 per cent, with a few affected plants only in some fields and severe injury in others (Fromme, Wingard, and Priode, 1927). Practically all of the leaves on some plants may be affected, while others show only a few scattered lesions. When the leaves are severely infected, they are, at maturity, light in weight and poor in quality.

It seems quite probable that ring spot has been de-

scribed under other names. It appears to be identical with *pokziekte* first described in 1890 (Iwanowski, D. and Poloftzoff, W. Mem. Acad. Sci. St. Petersbourg. 7 ser. 37, 7: 1890). It seems also to be identical with Selby's (1904) Figure 1 labelled "tobacco leaf showing mosaic disease."

Cause.—The evidence in hand indicates that the cause of ring spot is a virus. This has been determined by failure to isolate in culture organisms, either bacteria or fungi, from infected leaves, coupled with the fact that the juice from diseased leaves is infectious. When such juice is applied with a swab to healthy leaves not yet mature, infection develops three to five days afterward.

No infection was produced when an inoculum was prepared from diseased leaves which were air dried for a month (Priode, 1928). Juice from diseased plants loses its virulence very rapidly at room temperature, being innocuous in about twenty-four hours, but is infective eighty-five days after extraction when stored at 5° C. Apparently the duration of the retention of virulence in expressed juice varies inversely with the temperature at which it is stored. The infectious principle does not pass through a Berkefeld filter of grade "N" (Priode, 1928). The inoculum used in the inoculation experiments of Priode (1928) and Wingard (1929) consisted of the sap of infected tobacco leaves, diluted about one to ten with water. This was applied immediately after being prepared, with a swab, to the surface of the plant to be inoculated. The inoculations were made in late afternoon, and no effort was made to protect against the drying of the inoculum. The large number and proportion of infections which followed inoculation on a wide range of suscept species leave no doubt that an infectious principle exists in the juice of diseased plants. In other cases needle-prick inoculations were made.

Price (1932) noted that certain kinds of tobacco make a more or less complete recovery from ring spot. When the stem tips of these recovered plants are rooted and have grown into new plants, no symptoms appear when

they are subsequently inoculated. This he interpreted to show that the plants had acquired immunity to ring spot. He found that the immunity could be communicated through three generations of Turkish tobacco cuttings. Seedlings grown from seed from these "immune" cuttings, however, were susceptible to ring spot. Again, the sap from the recovered cuttings was highly infectious to healthy tobacco plants. Valleau[5] pointed out that the leaves which failed to develop the ring symptoms, do present other symptoms, and therefore must not be regarded as being "immune." Their cells are completely invaded and occupied by the virus, and the matter of acquired immunity is in no wise involved.

Contributory Factors.—Too little is definitely known about how the ring spot virus survives the winter and about the agencies involved in its dissemination during the growing season. Such perennial-rooted plants as horse nettle and poke weed, which commonly occur around fields or seed-bed sites, are known to be highly susceptible to ring spot and might therefore play a part in its overwintering. Valleau (1932) regards horse nettle as a common carrier, and transferred ring spot from this weed to tobacco. Natural infection has been noted to be rather common on sweet clover (Wingard, 1929). Furthermore, when tobacco follows clover or alfalfa in rotation, ring spot is often very severe. These observations suggest that leguminous crops are in some manner responsible for the presence of ring spots on tobacco.

Recent investigations by Henderson (1934) contain evidence that the virus overwinters in sweet clover. He points out, however, that it is not established that the virus of tobacco ring spot is identical with the sweet clover viruses because the sap expressed from tobacco plants affected with ring spot has not been shown to be infectious to sweet clover. If the sap from affected sweet clover plants was used to inoculate tobacco, symptoms similar to ring spot developed. He states in explanation

[5] In a paper presented before the American Phytopathological Society, December 27, 1934.

that the viruses from sweet clover may be forms or strains of the tobacco ring spot virus.

The systemic nature of the disease indicates that it might occur within the seed and that the planting of infected seed would be a means of initiating the disease in succeeding seasons. Valleau (1932) has demonstrated that both the green strain and the yellow strain of ring spot virus are seed-borne. The distribution of ring spot-affected plants in the fields is not of the random character to be expected if it were transmitted by seed, but suggests (Valleau, 1932) insect carriage of the virus from perennial plants. Fenne (1931) was unable to transmit ring spot by means of such common insects in tobacco fields as tobacco flea beetle (*Epitrix parvula* Fabr.) and tobacco worm (*Phlegethontius quinquemaculata* Haworth). Henderson (1931) saved seed from petunia plants that had been inoculated with ring spot virus from tobacco, and among 914 seedlings he secured 181 affected seedlings.

Control.—No means of control based on definite experiments are available. Since the field observations indicate that the prevalence of ring spot is related to the growing, during the previous season, of a leguminous crop, rotations for tobacco must be planned to guard against tobacco following legumes immediately in the rotation system.

The destruction of such susceptible weeds as horse nettle and poke weed in the vicinity of seed-bed sites is to be recommended as a preventive measure.

Seed from ring spot-infected plants should not be saved for planting.

Spread of the disease from plant to plant, which results from the handling of diseased and healthy plants promiscuously during the growing season, may be avoided.

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ROTTERDAM-B DISEASE OR CURLY DWARF

The Rotterdam-B disease was so named because of the fact that it was first noted on the Rotterdam-B plantation in Sumatra, in 1926. Since then, according to Jochems (1926, 1928), it has appeared every year on most of the plantations of the coastal plains of Deli. It is not of serious importance, however, because it does not involve all plants in any portion of the field; but affected plants occur here and there, sometimes widely separated. The disorder appears to be identical with one noted by Slagg (1926) in 1922 in Connecticut, and subsequently in Ontario and Wisconsin on varieties of cigar-tobacco. Apparently, too, it is the same as the disease recently described from Baden, Germany, by Böning (1931), as *Streifenkrankheit* (streak disease) or *Kräuselkrankheit* (curl disease).

SYMPTOMS.—Apparently plants ranging in age from very young ones to those that are practically mature are subject to attack. The first evidence of disease on young plants is a mottling of the top leaves, upon which pale green to yellowish green areas develop. These have no sharply delimited border, but merge gradually into the surrounding normal tissue. Small necrotic brownish spots soon appear, about 5 mm. in diameter. The lesions tend to be angular because they are limited by the smaller veins. Gradually affected leaves become entirely yellow,

their tips droop, and the necrotic areas enlarge to become large dry areas.

In most instances affected leaves are somewhat distorted, and their margins are undulate or else rolled downward because of the occurrence of dark necrotic lesions on the veins. The death of the vascular tissues may involve the entire midrib and veins; and, in consequence of this involvement, the leaves fade and die. Ob-

FIG. 53. Terminal portion of the stem of a plant affected with Rotterdam-B disease. Necrotic lesions occur on the leaves, and the vascular tissues are discolored. (After Jochems.)

servations of plants in the field show that fully grown leaves are either not attacked or else the mottled condition characteristic of early infection fades out. Furthermore, not all leaves on affected plants may exhibit symptoms, but usually two or three of them only.

When young plants are attacked, dark layers of cells may appear in the stems, involving the cortex, vascular ring, and pith. This necrotic condition resembles stipple streak in potatoes. It begins internal to the stem, and is faintly evident at the outside for some time in advance of involvement of the cortical tissues. Finally the whole stem turns black, and the plant dies. In less severe cases the plants may be so stunted as to be about half the size of near-by normal ones.

Cause.—The sap of diseased plants, when passed through a Berkefeld or Chamberland filter, is still infectious. The greater part of the infectious principle, however, is retained on the filter. Then this filtrate, when atomized onto healthy plants, after a seven days' incubation, results in symptoms like those on plants from which the sap was extracted. This shows that the causal agent is one of the viruses. Transfer of inoculum consisting of juice from crushed affected leaves results in infection in nearly 100 per cent of cases, when the sap is applied by rubbing upon young top leaves. The grafting of affected parts to healthy stems resulted in the spread of the disease to the stocks. Dried diseased leaves were still infectious after nine months.

Jochems (1928) found that the three strains of Deli tobacco used in his inoculation studies were equally susceptible to the Rotterdam-B disease. His inoculations, in addition, on *Nicotiana sylvestris* resulted in infection. He also inoculated tomato, pepper, eggplant, and two solanaceous species of weeds, *Physalis angulata* and *Solanum torvum,* but no infection developed upon any of these plants.

Control.—So little is known about the disease that definite recommendations for control based upon experimental evidence cannot be given. It appears probable

that the virus survives from season to season in tobacco refuse in the fields. The virus, moreover, is probably transferred from diseased to healthy plants by contact. Sanitary measures directed toward its exclusion from the fields, and toward its early eradication when affected plants are found in the field, are undoubtedly basic to control. Böning (1931) found no evidence that it is seed-borne, but noted that the disease may appear in the seed beds and be carried to the field at time of transplanting. His observations indicate that spread of the disease in the field is the result of handling plants during cultivation.

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Kroepoek or Leaf Curl

Our early knowledge of this disease comes from the observations of Ludwigs (1913) in the Cameroun, West Africa, and of Jensen (1920) in the province of Vorstenland, Java. It had been observed prior to this, however, and is mentioned in Peters and Schwartz's treatise (1912) on diseases and insect pests of tobacco. They state that it occurs in Java, Sumatra, and is probably present in Ceylon. Thilliard (1921) investigated this disease in Cameroun for several years, and applied to it the name "leaf curl." More recently Storey (1932) at Amani, East Africa, and Thung (1932) at Klaten, Java, almost simultaneously discovered the cause. Kerling's (1933) account contains a description of the anatomical changes which characterize kroepoek.

Suscepts.—In view of the fact that the cause of kroepoek is communicated by the white fly, *Bemisia gossypiperda*, while feeding, and that the food plants of this insect are not yet completely known, one cannot know the suscept range. Cotton is affected with a leaf curl disease caused by the same virus that causes kroepoek, and the virus is transmitted by the same species of white fly. Apparently one or more species of *Vernonia* should be included among the suscepts, together with Biloxi soybeans, *Crotolaria usaramoensis*, cassava, wild tomato, and *Canavalia* sp., as reported by Storey (1932). Ludwigs (1913) ventures the opinion that elephant's ears, *Colocasia antiquorum*, is subject to the same disease. Kerling (1933) noted it on garden zinnia, *Zinnia elegans*, and Thung (1932) induced symptoms of kroepoek on tobacco following transfer of white flies from diseased zinnias to tobacco. More recently he (Thung, 1934) reported that the host plants near Klaten, Java, include such weeds as *Ageratum conyzoides*, known there as *bandotan* or *wedversan*, *Synedrella nodiflora*, called locally *legetan* or *totowok*, and *Vernonia cinerea*, known locally as *Njawon*. *Datura* sp. is also believed to be a weed host. His report is illustrated with photographs of the disease on *Nicotiana glauca*, *N. rustica*, tomato, *Zinnia elegans*, and *Synedrella nodiflora*.

Symptoms.—Apparently the name *kroepoek*, also spelled *krupuk*, is derived from the Dutch word *kroes* (meaning to curl or to frizzle), since this term describes the appearance of affected leaves. Jensen (1920) says the leaf appears if "kneaded and wrinkled in the hand, and again partly unfolded from itself." The word may perhaps be derived from the name of spices added to rice as prepared for food in the Orient. It has also been called *krulziekte* in the East Indies, and appears to be identical with *faltenzweig* in Dalmatia, and *narrischwerden* (becoming foolish) in Germany. Jochems (1926) description of *gilah*, in Java, indicates that this condition is identical with kroepoek. He noted that diseased and healthy plants might occur in the same row near each

FIG. 54. Leaves deformed by kroepoek or leaf curl.
(After Moore.)

other and that there was no evidence that the disease was seed-borne. Among the names employed for this disease are *krepoh*,[6] in Java, and *crinkle*,[7] *Kroesblaar*,[8] or crinkly dwarf,[8] in South Africa.

Kroepoek has not been observed on seedlings, but may appear on young plants in the field soon after they have been set. Diseased plants may occur irregularly distributed throughout the field, but occur earliest and most abundantly near the borders of the fields, or near the villages, or barns where 50 to 70 per cent of the plants may be involved. If young plants are affected, they never recover, and the entire plant may be dwarfed to one-third or one-half the normal height. When older plants are affected, the lower leaves may be normal, and those in the tops may be diseased. The flowers may also be misshapen, as shown by Jensen (1920).

Thung (1932) described three forms of the disease: (1) common kroepoek, (2) curl disease or crinkle disease, and (3) transparent kroepoek. In common kroepoek the leaf-tip and leaf-margin are rolled downward, the small veins are irregular with knob-like thickenings, and the larger veins are very prominent and bear secondary blades or lamina from one-half to three-quarters inch long. The backs of these secondary blades face the backs of the main leaf. Oftentimes cup-like outgrowths or ascidia form along the midvein and are joined to the main leaf with a stalk that is petiole-like in appearance.

In the curl disease the stems are branched, and the entire leaf is rolled downward along the margin. The midrib is not contorted, and the veins are uniformly thick-

[6] KEUCHENIUS, P. E., Waarnemingen over het ziekten en plagen bij Tabak. MEDED. BESOEKISCH. PROEFSTAT. **14**: 12. 1915. GANDRUP, J., Verslag over het jaar 1923 door Dr. W. H. Arisz. MEDED. BESOEKISCH. PROEFSTAT. **36**: 20. 1924. SCHWEITZER, J., Verslag over het jaar 1925 door Dr. W. H. Arisz. MEDED. BESOEKISCH. PROEFSTAT. **41**: 14. 1925. ———, Over Virusziekten bij Tabak. VERSLAG NEGENDE VERGRADERING V. V. P. P. p. 81. 1928.

[7] ROBERTS, J. I., The tobacco capsid (*Eugytatus volucer* Kirk.) RHODESIA BUL. ECON. RES. **21**: 169. 1930.

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ened. The intervein tissues convexly curve upward. This form of the disease is most prominent on the top leaves.

In the transparent form of kroepoek, the leaf-margins are rolled upward. The midrib and lateral veins are shortened and appear as if their normal elongation had been forcibly impeded, "as if they are somewhat laced by a thread" (Thung, 1932). The small veins are transparent. Knotting of the veins and the formation of secondary blades do not occur in this form of the disease.

Kerling (1933) is of the opinion that common kroepoek and transparent kroepoek are distinct, and that they may be caused by distinct viruses, even though both are transmitted by the same species of white fly.

Kerling's anatomical studies show that in kroepoek there is an increase in the phloem of the veins, an enlargement of the pericycle through the formation of new cells, a formation of new woody cells, and the initiation of new steles inside the old pericycle. Tissues in the new steles are in reverse orientation, and the morphological lower side of the new leaflets is directed toward the lower side of the old leaf.

Cause.—Kroepoek is another of the virus diseases of tobacco, and is transmitted by the white fly, *Bemesia gossypiperda*. This fact was announced by Storey (1931) and by Thung (1932) from independent investigations, the former in Africa and the latter in Java. This insect is merely the vector and disseminates the virus while feeding. This fact was established by introducing viruliferous white flies into insect-proof cages in which tobacco was growing with the result that such plants became kroepoek-affected, while plants similarly grown and kept free of white flies remained normal.

Prior to the investigations by Storey (1931) and Thung (1932) the cause was suspected of being some water relation, for the reason that the spring crop, grown in the dry season, was badly affected in Cameroun, while the fall crop, grown in the rainy season, was normal (Ludwigs, 1913). That it was not a simple water relation was shown, in his experiments, by the fact that to-

bacco plants could not be made to recover when supplied with water.

CONTROL.—Measures for control have not been determined by experiment, except in Java (Thung, 1934). Manifestly, control is contingent upon eradication of the white fly, or of its food plants. It would appear to be feasible to carry out these operations. Thung shows that the white flies come from the weed hosts that thrive on the borders of the native villages and surround the tobacco fields. Very satisfactory control followed the destruction of the weeds in these situations for a distance of 50 meters from the borders of the tobacco fields. The weeds were removed three times during the season, in mid-June, in mid-July, and in mid-August.

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TOBACCO WITCHES' BROOM

This disorder has never been noted in nature and is of no economic importance. It has been produced under experimental conditions by transmission of the infectious principle through grafts from potatoes and tomatoes on which a witches' broom disease is of rare occurrence in several of the states of the western United States.

Suscepts.—In his preliminary report Young (1928) showed that it is possible to communicate this disease to tobacco from affected potatoes or tomatoes, and from tobacco thus artificially inoculated back to tomatoes. He also attempted, but without success, to inoculate ground cherry (*Physalis* sps.). Nothing further is known about the susceptibility of other members of the family *Solanaceae*.

Symptoms.—The symptoms of witches' broom consist of the presence of numerous, dwarfed, spindling branches, both basal and in the leaf axils above. The leaves borne on such branches are themselves much smaller than normal, and the leaf margins are abnormally light green to yellow.

When affected tissues are examined under the microscope, the palisade tissues are found to be abnormally broad and of less than usual length. The intercellular spaces are abnormally prominent.

Cause.—The causal agent of witches' broom is an ultra-microscopic principle called a virus. That it is distinct from other viruses that occur within tobacco is determined by the fact that it produces distinct symptoms. Like other viruses, however, it is able to increase within living tissues, and is presumed to have physical and biochemical properties quite like those of the ordinary tobacco mosaic virus.

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Young, P. A., Tobacco witches' broom. A preliminary report. Amer. Jour. Bot. **16**: 277-279. 1929.

The Delphinium Virus Disease on Tobacco

The studies by Valleau (1932) constitute the only investigation dealing with a virus disease first noted on Delphinium but communicable to tobacco by rubbing. Subsequently it was collected on tobacco growing in the fields in Kentucky and in Minnesota.

Symptoms.—The first evidence of disease on Turkish varieties of tobacco is a necrosis appearing in fine rings and lines. Leaves that form subsequent to inoculation

may be normal, or the chlorotic patterns may be so faint as to be evident only when the leaves are examined by transmitted light. When, on Turkish tobacco, extensive necrotic patterns appear resembling those of coarse etch, the next leaves to unfold may be distorted. On White Burley tobacco grown in Minnesota, the disease is characterized by irregular, ring-like, orange-yellow, chlorotic patterns, that may be bordered by a fine necrotic line or by dots, suggestive of ring spot. The two disorders would not be confused, however, by one who had become familiar with ring spot as the result of experience in the field.

Suscepts.—Valleau (1932) was able to communicate the virus from Delphinium to Turkish tobacco and to White Burley tobacco with the production of symptoms like those just described. He also transmitted virus from White Burley tobacco to Delphinium, where it produced symptoms indistinguishable from those originally noted on Delphinium. A conspicuous mosaic disease on cucumbers resulted from inoculations with virus from Delphinium, or with virus from White Burley, from Kentucky or from Minnesota. He suggests that its properties indicate that it corresponds most closely with the cucumber-mosaic viruses.

Control.—Studies on the possibility that this virus may be seed-borne have failed to give positive results. Up to the present, the observations on this virus disease have been too limited to indicate any means of control.

Reference

Valleau, W. D., A virus disease of Delphinium and tobacco. Ky. Agr. Exp. Sta. Res. Bul. **327**: 81-88. 1932.

Kromnek or Crook-Neck

Lounsbury (1905) described a disease of tobacco in the Kat River Valley of South Africa as a "wilt" that has been presumed to be caused by a species of *Fusarium*. This conclusion as to the cause was drawn by Moore (1925) in a report published in 1925, but in more recent investigations (Moore, 1933) she presents evidence that

the disease is caused by a virus. The writer is of the opinion that the disorder which was described from Argentina (Fawcett, 1921) as *corcova* is identical with kromnek. The *jaroba* disease, thought by Spegazzini (1898) to be a manifestation of downy mildew, may also have been this kromnek disease.

Symptoms.—The disease may appear both on seedlings and on more mature plants in the field. Its most characteristic symptoms include a pronounced stunting and deformation. In the case of seedlings, the development of the entire plant may be inhibited. In older plants, the basal leaves may be normal, but those near the top of the plant appear puckered or "gathered." This puckering is the result of cessation of elongation of the midrib, whereas the tissues of the blade continue to grow at a rate approximating normal. If the growth on one side of the midrib is inhibited more than that on the other, the leaf becomes twisted, and the underneath surface may then be directed upward. The top of the plant may be bent so as to nod, or to be twisted or looped when the apex of the stem is involved. In the Virginian tobaccos, that are more susceptible than Burley tobacco, sunken streaks may be formed. They usually appear as a late symptom, and there may also be present at this time discolored cavities in the pith.

Suscepts.—Moore (1933) noted that the disease not only appears on tobacco but on a number of other solanaceous species, including *Datura stramonium*, *Nicandra physaloides*, *Physalis minima*, *P. peruviana*, and *Solanum nigrum*. As the result of artificial inoculation, it has been communicated to tomato, potato, and *Solanum pseudocapsicum*.

When affected tobacco plants were grafted to healthy tomato plants, symptoms of a disease resembling "bunchy top" of tomatoes appeared on the new growth from the stocks. On the other hand, when bunchy top tomatoes were grafted to healthy tobacco plants, the kromnek disease developed on the new growth from the tobacco. These experiments lead to the conclusion that the cause

of kromnek of tobacco is identical with that of bunchy top of tomato.

Cause.—The experimental work on this portion of the problem shows that kromnek is due to a virus. The virus appears to be spread by an insect, a species of *Frankliniella*. Spread appears to be checked by high temperature, but whether this is a direct effect of temperature on the virus or on the vector is not established. There is no evidence that the virus is seed-borne.

Control.—Measures for control are unknown. The fact that the incidence of the disease is influenced by warm weather suggests that late planting may be a means of avoiding serious injury from kromnek.

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Other Virus Diseases

The virus diseases of tobacco that hitherto have been considered produce sufficiently characteristic symptoms on tobacco so that the initiate in the problem of virus diseases soon learns to recognize them. A number of others, however, have been described that are less easily recognizable as specific entities, partly because of the lack of a satisfactory basis for the classification of the infective agents, and, as a result, the virus disease problem is confused. The investigations on virus diseases of tobacco, however, clearly show that a number of different virus diseases exists, some of which occur on other species of plants upon which they produce distinctive effects. When tobacco is again inoculated with virus from one

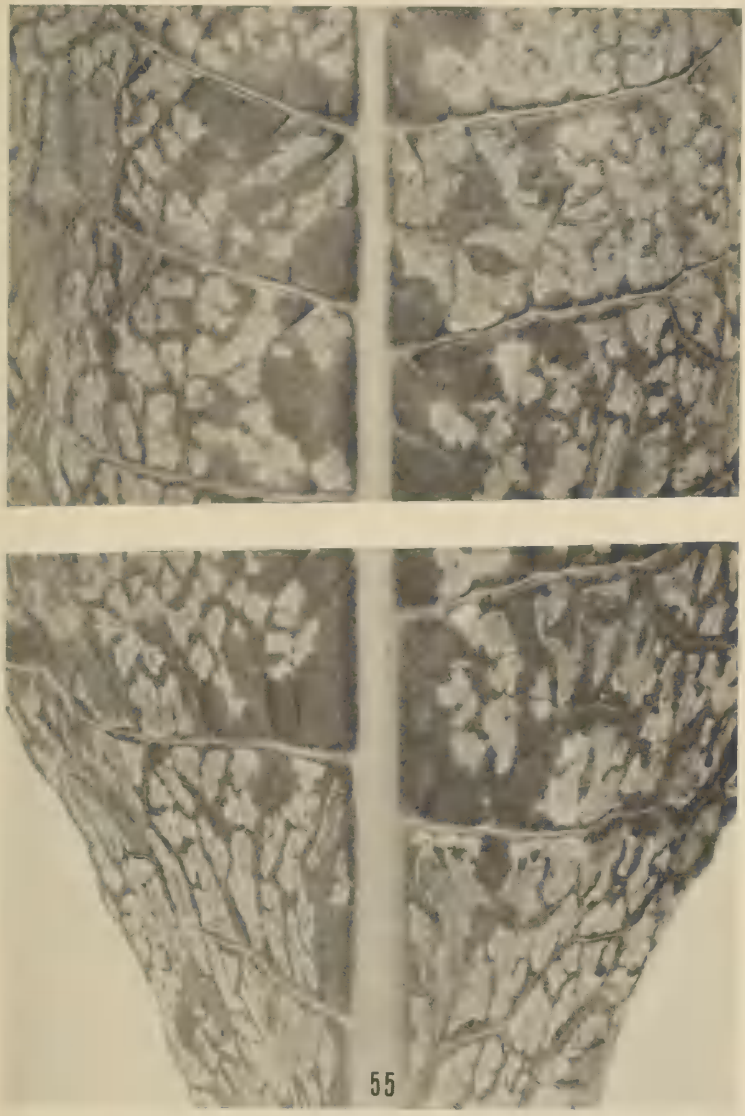

FIG. 55. Yellow mosaic on Burley tobacco showing irregular, lemon-yellow areas that involve the tissues between the veins. (From E. M. Johnson.)

of these other suscept species, the symptoms produced in tobacco are the same as if the virus had been repeatedly used to inoculate a series of tobacco plants. This indicates that the properties of the virus are not greatly modified by the suscept species into which it is inoculated.

Many virus diseases are so commonly associated in nature with a specific plant and can be communicated to the tobacco plant that it is logical to designate them by the name of the suscept on which they normally occur. This is the case, for example, with cucumber mosaic, that may readily be transferred to tobacco.

The status of the tobacco-virus-disease problem may be appreciated from the studies of James Johnson (1927) who recognized 11 different viruses on tobacco and related plants, of Fernow (1925), who described 8, of McKinney (1929), who described 3, and of E. M. Johnson (1930), who described 18. The accounts both of James Johnson and of E. M. Johnson contain keys for the identification of these different diseases. The latter makes 7 groups of the virus diseases that occur on tobacco. One of these includes the true tobacco mosaics which occur only on solanaceous plants and whose viruses retain their power of producing infection after long periods of desiccation. The other 6 groups are the cucumber mosaics, etches, coarse etch, veinbanding, ring spot, and "healthy" potato virus disease. None of the viruses that cause these disorders withstands air-drying, and some affect plants belonging to species other than the *Solanaceae*.

Sometimes mixed infections occur on tobacco in the field so that the symptoms may be confusing even to the specialist. Not only can it be demonstrated that mixing occurs, but in some cases the viruses can be separated. If, for example, tobacco is affected with both cucumber mosaic and ring spot, the viruses may be separated if sap from the affected tobacco plant is used to inoculate both cucumber and tomato. As a result of such inoculations, since tomato is not susceptible to ring spot, only cucumber mosaic will develop on tomato, whereas both diseases will appear on the inoculated cucumbers. In the

same way a mixture of cucumber mosaic virus and veinbanding virus may be separated with cucumber plants as the differential medium, because cucumber is not susceptible to veinbanding. Again, a separation of the etch viruses from the ordinary tobacco-mosaic virus can be effected by inoculating Jimson weed (*Datura* sps.).

A brief summary of the more important information regarding these less well-known mosaics follows:

Yellow Mosaic.—This mosaic is manifest by the formation of large, irregularly angular, lemon-yellow areas that occur along the veins. The bud leaves are twisted and distorted. Following inoculation, there is first a clearing of the veins, accompanied by rugoseness of buds, and then the leaves become mottled. After seven to ten days the yellowing appears. Plants grown under glass in winter, if inoculated, may not become yellow, and under these conditions the symptoms resemble those of the ordinary tobacco mosaic (Type 1). Brown necrotic areas may occur on mature leaves in the field, and these areas may eventually fall away. When virus from yellow mosaic is inoculated into *Nicotiana glauca,* circular, white chlorotic areas form; into Petunia, irregular white to yellow areas.

Recently Jensen (1933) isolated three groups of yellow-mosaic viruses from yellow spots on Turkish tobacco, *Nicotiana sylvestris,* and tomato that had been inoculated with typical tobacco-mosaic virus. Those of the first group were systemic and easily communicable. They produced symptoms that were quite like those of typical mosaic except that there was more intense yellowing, and they caused the clearing of the veins in tobacco. Those which produced the most intense yellowing oftentimes caused the death of affected tissues. One of this group of viruses produced lesions which inoculated into tomato, eggplant, *Nicotiana sylvestris, N. glauca,* and *N. paniculata.* It produced local necrosis in *N. glutinosa, N. acuminata,* and *N. sylvestris,* and systemic necrosis in *N. rustica* and *Physalis angulata.* Its symptoms appear

similar to those of Tobacco Mosaic No. 6 of James Johnson (1927), White Mosaic of E. M. Johnson (1930), and Aucuba Mosaic as described by Smith (1928).

The yellow-mosaic viruses of the second group produced small yellow spots but with no clearing of the veins.

FIG. 56. Yellow mosaic of Connecticut-Havana tobacco on leaf and stem, the result of artificial inoculation. The oak leaf-like pattern follows each of the principal veins. The dark patch near the tip is normally green. (After McKinney.)

Leaves formed subsequent to inoculation had ring-like blotches that enlarged slowly until the entire tobacco leaf was yellow.

Some of those of the third group produced few or no yellow spots except on the inoculated leaves. Others formed oak-leaf patterns on the lowermost leaves.

Neither those of the second nor of the third groups gave high percentages of infection when undiluted virus extracts were used for inoculation. Jensen believes that the yellow-mosaic viruses are to be regarded as strains of the typical or ordinary tobacco-mosaic virus, and that they have arisen from it.

Cucumber Mosaic.—This mosaic occurs most commonly on cucumber and related plants, but in some sections it is rather common on tobacco in the field. Johnson (1930) recognizes three types of cucumber mosaic, but all of them cause tobacco foliage to have a generalized chlorosis, with occasional dark green, puffed areas. Cucumbers, when infected, are severely stunted, and the leaves and fruits are malformed. Generally there is little malformation in cultivated tobacco infected with cucumber mosaic, but *Nicotiana glutinosa*, if inoculated, is stunted and malformed.

The first visible symptom on tobacco consists of the appearance of faintly pale green areas in the intercostal areas.

Pokeweed *(Phytolacca decandra)* becomes mottled when inoculated with cucumber-mosaic virus.

Veinbanding.—This type of mosaic occurs in Kentucky on Burley tobacco that is grown in or near potato fields, and the virus may be readily obtained from mottled potatoes. It is identical with the potato virus y of Smith (1934) that causes the stipple streak of potato. Veinbanding of tobacco is characterized by narrow bands of dark green tissue that appear along the veins. Sometimes veinbanding is visible only in the lobes at the base of the tobacco leaf. Minute chlorotic spots may form between the larger veins in young leaves, and these spots become

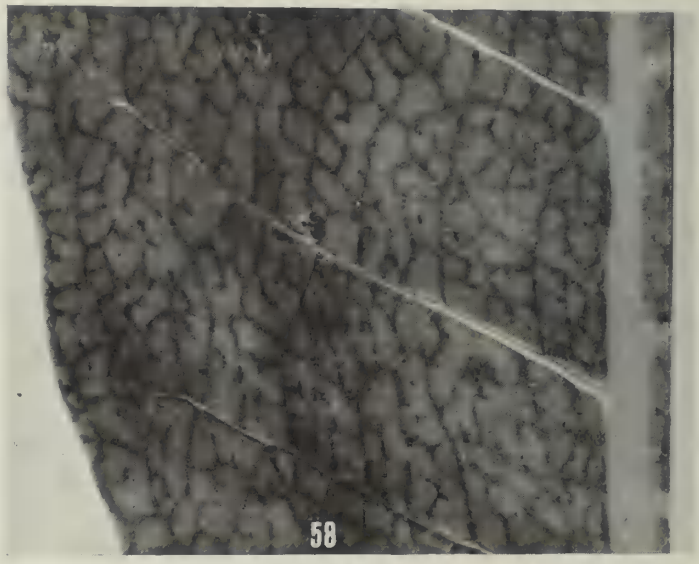

FIG. 57. Severe etch of Burley tobacco leaf. The midrib and veins are necrotic. (From E. M. Johnson.)

FIG. 58. Veinbanding of a Burley tobacco leaf as shown three weeks after inoculation. The tissues adjacent to the veins appear as dark bands or tracings. (From E. M. Johnson.)

FIG. 59. Mottling of Burley tobacco leaf following inoculation with the virus of cucumber mosaic. It may be confused with ordinary or typical tobacco mosaic. The light areas tend to be more definite, however, and to be more yellow. (From E. M. Johnson.)

large, indefinite-margined, yellow areas on the older leaves.

Burnett (1934) has shown that the tissue of tobacco and potato from veinbanding-virus-infected plants, after having been dried for fifty days, is still infectious. The virus was able to tolerate drying in tomato tissues for only seventeen days. During this desiccation the plant tissues were maintained at a temperature of 67° to 75° F.

Etches.—Johnson (1930) distinguished four types of etches, namely, coarse etch, severe etch, etch +, and etch, whose differences are easily seen when direct comparison is made of plants inoculated with one or the other of these four types. The most pronounced symptom is the presence of circular or arcuate, necrotic areas that are bounded by chlorotic areas. Older leaves of infected plants develop vein necrosis; younger leaves, especially the bud leaves, are wrinkled and distorted.

"Healthy" Potato Virus Disease.—This disorder is not known to occur on field-grown tobacco. When the sap from apparently healthy potato leaves is applied by rubbing to tobacco plants, small, chlorotic spots, about one-sixteenth inch in diameter, with a fine necrotic border, may develop within four to twenty-one days. Subsequently similar rings and line patterns, which resemble the water marks on bond paper or those on watered silk, appear. These may best be seen by transmitted light. This disease is apparently like the ring spot of tobacco (Tobacco Virus 5), described by Johnson (1927), and the potato virus x as designated by Smith (1934).

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VII

Bacterial Diseases of Tobacco

Introduction

That plants are subject to bacterial diseases was first demonstrated in connection with fire-blight of pomaceous fruits (apples, pears, etc.) in 1880. Since that time a large number of bacterial diseases of plants have been described. Smith (1920) reported that plant pathological literature records the occurrence of bacterial diseases on more than 150 genera of plants. Vast areas of the world are wholly or almost wholly unexplored for bacterial diseases of plants.

No definite date can be set as to when our knowledge of bacterial diseases of tobacco began. Nearly all of it has been acquired, however, within a generation. Granville wilt or slime disease is the first one to be investigated comprehensively. A number of diseases of tobacco of bacterial origin have been described, and at present no one knows how many of these are distinct. The only special investigation that has been directed toward a clarification of this matter was that of Stapp (1930). He considers that there is little doubt of the identity of *Bacterium melleum* that causes the Wisconsin bacterial leaf spot disease, and *Bact. tabacum* that causes tobacco wildfire. The former of these, however, produces yellow colonies, on agar, the latter grayish white ones. Stapp also believes it is probable that black rust, caused by *Bact. pseudozoogloeae* and *Bact. tabacum,* are identical. The symptom complexes induced by these organisms, as shown in the literature of these diseases, are entirely distinct. He regards the knowledge of *Bact. maculicola,* the cause of white speck, as too meager to draw any conclusion, with which opinion the writer is in accord. Lastly, he regards

Bact. tabacum and *Bact. angulatum,* the cause of angular leaf spot, as without doubt identical. Here again the two organisms differ in their reactions on carbohydrates, as the present writer has been able to demonstrate, and in the symptoms of disease exhibited on tobacco. Stapp's (1930) paper contains a résumé of the morphological, cultural, biochemical, and physiological characters of the five above-named species of bacteria.

This lack of agreement on the specific limits of disease-producing bacteria on tobacco, or on other crops for that matter, will continue to exist until there is a standardization of technique for studying this group of organisms. At present it is largely impossible for two investigators to get similar results from cultural, biochemical, and physiological studies of any particular bacterium. The writer (1920) called attention to the cause of several of these difficulties and suggested ways of avoiding them. For example, carbohydrates are known to be hydrolyzed by heating in the presence of acids or alkali, and so should be sterilized in distilled water and added aseptically to sterilized cooled media. By the use of "rare" sugars it is possible to separate certain organisms that are very similar. The writer (1920) was able to separate *Bact. tabacum* and *Bact. angulatum* by the ability of the former to ferment mannit and galactose, neither of which are utilizable by the latter. Again, whether an organism is strictly aerobic can only be known if the fermentation tubes are left undisturbed during the entire duration of the tests; otherwise clouding may occur in the closed arm as the result of agitation. Colony characters on agar vary, dependent, among other factors, upon the viscosity of the agar. Percentage composition of agar must, therefore, always be recorded. Colonies of *Bacterium tabacum* and *Bact. angulatum* even in the same Petri dish culture differ in surface markings, character of the margins, and virulence. The existence of different strains of plant-pathogenic bacteria is a well-known phenomenon.

In the following synopsis of essential knowledge of the bacterial diseases of tobacco the writer has made no at-

tempt to determine whether identities exist. This can only be determined by a thoroughgoing, comparative investigation. Neither is an attempt made to follow the present-day system of nomenclature. This failure will not cause any confusion for bacteriologists, because of their acquaintance with the various systems, nor for other investigators and laymen.

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GRANVILLE WILT OR SLIME DISEASE

Granville wilt is a serious disease of tobacco in portions of the flue-cured district in North Carolina and Virginia, the cigar-tobacco district of western Florida and southern Georgia, and in Japan and the East Indies. It has been more extensively investigated than any other plant disease of bacterial origin with the possible exception of fire-blight of pomaceous fruits, but effective control measures have not been developed.

SUSCEPTS.—The organism that causes this disease does not confine its attacks to cultivated tobacco, *Nicotiana tabacum*, but occurs on other species of *Nicotiana* and numerous other species of plants. *Nicotiana rustica* from Italy, Russia, China, and elsewhere were found to be very susceptible. Among other species tested were: *N. longiflora*, *N. glauca*, *N. glutinosa*, *N. langsdorffii*, *N. sylvestris*, *N. affinis*, and *N. sanderae* (hybrid), none of which showed any marked resistance (Garner, Wolf, and Moss, 1917). In tests made by Stevens (1904), 62 types known by distinct varietal names were used. These included strains of Orinoco, Pryor, White Burley, Maryland Broadleaf, Havana Seed types of the northern cigar-leaf sections, together with domestic and imported Cuban, Turkish, and Sumatra tobaccos. In all tests of these domestic types 70 to 90 per cent of the plants succumbed to wilt.

In the tests with foreign varieties, including varieties from South America, Mexico, Cuba, Sumatra, Turkey, Italy, and Russia, it was found that the Sumatra, a Turkish variety from Cavala in Macedonia, and a variety from Italy known under the varietal name *fruticosa* showed considerable resistance. In the various hybrids which were produced the striking feature was the variation in resistance from year to year. A Cuban tobacco showed a resistance of 12 per cent in 1911, 96 per cent in 1912, 78 per cent in 1913, and 14 per cent in 1914, apparently due to varying seasonal factors (Garner, Wolf, and Moss, 1917). Honing (1910) tested varieties from various parts of the world, including 30 or more American varieties, and found none more resistant than the native Sumatra. Kuijper (1929) found *Nicotiana sylvestris*, *N. sanderae*, and *N. affinis* to be very susceptible.

Among other species of *Nicotiana* which have been found to be subject to attack are *N. tomentosa*, *N. suaveo-*

FIG. 60. Field of flue-cured tobacco affected with Granville wilt or slime disease. Some of the dead plants had been removed, and others remain. Plants whose leaves are wilted and droop usually die after a few days.

lens, N. macrophylla, N. quadrivalvis, N. atropurpurea var. *grandiflora,* and *N. alata* var. *affinis.*

In addition to tobacco, the organism attacks such cultivated plants as banana, tomato, eggplant, pepper, Irish potato, watermelon, sweet potato, peanuts, beans, peas, soybeans, cowpeas, and velvet beans. That peanuts are subject to attack by the same organism was first found by van Breda de Haan (1906) in the East Indies, and by Fulton and Winston (1913) in North Carolina. Its occurrence on various weeds was noted by Uyeda (1905), when he inoculated *Physalis minima, Polygonum tinctorium,* and *Amaranthus gangeticus,* and by Honing (1910) when he reported its occurrence on *Ageratum conyzoides, Physalis angulata, Spilanthes acmella,* and *Pluchea indica.* Subsequent studies by Stanford and Wolf (1917) showed members of nine families of phanerogams, including over 50 suscept species, to be subject to attack by the tobacco-wilt organism. A number of the species in their list are weeds, such as ragweed, *Ambrosia artemisiifolia,* and fleabane, *Erigeron canadensis,* which are of common occurrence in tobacco fields in the flue-cured-tobacco districts. More recently Palm and Jochems (1922) list 34 species of wild plants, in Sumatra, occurring in 19 families of phanerogams, as subject to attack. In a later list of suscepts which they prepared (1924) are 125 species of plants. Among the best-known cultivated flowers that are susceptible to the Granville wilt pathogen are dahlia, cosmos, daisy, petunia, nasturtium, sunflower, and heliotrope.

The Disease

Names, History, Importance, and Range.—This disease within the United States was first designated "Granville Wilt" from the name of the county in North Carolina in which it was first definitely recognized (Stevens and Sackett, 1903). It was described in the same year by McKenney (1903), who regarded it as of fungous origin. Apparently "wilt," as it is now commonly called, had existed in Granville County, as recalled by growers, as

early as 1881. The disease appears to have been first investigated in Sumatra by Janse (1892), and later by van Breda de Haan (1898) and Honing (1910, 1914). They spoke of it as *slijmziekte* or "slime sickness." Uyeda (1905) notes that it was mentioned in a book on tobacco culture published in Japan in 1881, and is known there as "stem rot," "black leg," or "wilt," or such Japanese names as *tachigare-byo, ichōbyo,* or *kuromushi.* In Germany, it is known as *schleimkrankheit* and *schwartzbeinkrankheit;* in France, as *chancre bacterien;* in Hungary, as *slucak;* and among the Javanese, as *lier* and *lengker.*

In wilt-infested soils the disease is of serious consequence year after year, although no estimates are available of the total losses occasioned by it. Affected plants are killed outright, and a loss of 25 to 40 per cent in individual fields is not unusual in favorable seasons. Fields have been observed in which the crop was practically a total loss (Garner, Wolf, and Moss, 1917). In some areas the culture of tobacco on certain farms has been abandoned because of this disease. It has continued to spread from the time of its first appearance in Granville County, and is now known to occur in serious proportions in Durham, Vance, and Wake counties in North Carolina and to be present in small amount in about a dozen other counties. In recent years it has become an important menace in parts of South Carolina. Outside of the United States, Granville wilt or slime disease is known to occur in Argentina, Austria, Australia, British West Indies, British Guiana, Chosen, India, Japan, Java, Malaya, Mexico, New Zealand, Philippines, Russia, Sumatra, South Africa, and Trinidad.

Symptoms.—The disease affects the entire plant at any stage of development, but is usually first noticeable two to four weeks after transplanting. Following this there is a progressive increase in the number of wilted plants in any field until topping time, when dead and dying plants may occupy considerable areas.

Wilt can first be detected by a drooping of one or more

leaves of the plant. Those familiar with the disease can detect its onset, since wilting is not prominent for several days and the plants recover turgidity at night. The leaves soon become pale green, especially between the principal veins, and then gradually become yellow. Meanwhile the midrib and veins become limp and droop in a characteristic umbrella-like manner. This shape persists as the leaves become brown and crisp.

If the stalk of a wilted plant is cut across in early stages of the disease, yellowish discoloration of the xylem in streaks is apparent. These streaks darken to brown or black. In advanced stages the pith and cortex are affected; and dry, blackened areas appear which extend to the surface of the stalk. These streaked discolorations are very characteristic; and one may be certain of his field diagnosis if by pressure on such stalks, a dirty-white ooze or slime is forced out from the cut ends.

The root system is involved in decay also. This eventually results in the destruction of the smaller roots, probably by the invasion of secondary organisms, and the entire root system becomes involved, by the advance of the disease from the smaller roots toward the larger ones (Wolf and Moss, 1919).

Microscopic examination of the tissues of affected plants shows that the wilt organism occurs in the xylem vessels. The method of entrance is not known with definiteness, but presumably entrance is gained through the smaller roots, thence it passes into the larger, and finally into the main stem and skeletal tissues of the leaves. Entrance to the smaller roots occurs even though there has been no injury as from transplanting, cultivating, or attacks of nematodes. The bacteria multiply in such numbers within the xylem as to occlude the vessels and thus interfere with the water supply to the leaves. With the death of the plant the woody tissues and cortex are decayed, a process which is pretty certainly due mainly to secondary invaders.

Nakata (1927 a) was able to demonstrate that wilting is primarily due to the production of toxins, and that oc-

FIG. 61. Necrosis of veins, beginning at the leaf margin, due to the slime disease bacterium. This symptom occurs on affected plants in Japan. (From Nakata.)

clusion of the vessels is a secondary cause. He also attributed the browning and blackening of the xylem to the action of the enzyme tyrosinase on tyrosin, produced by the decomposition of albuminous substances in the tobacco plant by the pathogens.

Cause.—Tobacco wilt is caused by *Bacterium solanacearum* E. F. S. This is a well-known organism first studied by E.F. Smith (1896) in connection with a destructive rot of potatoes, eggplants, and peppers. McKenney (1903) believed it was caused by a species of *Fusarium,* one of the species of which was subsequently shown by Johnson to cause a wilting of tobacco. Stevens and Sackett (1903) did not assign a name to the bacterial organism which they found in their early studies of the disease. They noted, however, that the vessels of affected plants were filled with myriads of bacteria and stated that it is "strongly presumptive that these germs cause the Granville Wilt." Moreover, they mention a number of current notions held by growers as to the cause. These include fertilizers, improper crop rotation, climatic conditions, tillage, insects, and conditions of the seed bed. These are factors which influence the progress and extent of the disease, but the specific bacterium, above-named, is the primary cause.

Uyeda (1905) ascribed the wilt of tobacco in Japan as due to *Bacillus nicotianae,* which he described as a new species. The studies of Smith (1914), Honing (1910), and others have shown that the bacteria described by Smith and Uyeda are identical.

Pathogenicity.—A large number of workers including Smith, Stevens and Sackett, Honing, van Breda de Haan, Jensen, Jochems, Uyeda, Palm, Nakata, Fulton, Stanford, Bryan, and Wolf have demonstrated the pathogenicity of *Bacterium solanacearum.* If susceptible plants are transplanted to wilt-infested soils they will, in the course of time, succumb to attack. Moreover, if seed of tobacco are sown in infested beds, some plants may succumb before being set in the field, but usually the first evidence of infection may be noted about a month after

transplanting. In much of the inoculation work, the organism from pure culture has been inserted into stems through needle punctures. In general, as soon as wilting was first noted, the causal bacterium was reisolated, and its identity established by the characteristic growth on culture media, and by reinoculation into especially susceptible plants like tobacco and tomato.

Bacterium solanacearum is a rod-shaped organism, 0.5 x 1.5 μ, that possesses a single polar flagellum. It is Gram negative, aerobic, and does not form spores nor capsules. Colonies on agar are small, irregular, smooth, wet-shining, opalescent (white by reflected light, pale brown by transmitted light), becoming brown to dark brown because of a soluble pigment. Colonies on potato cylinders are white at first, and become dark brown. Gelatine is not liquefied, neither acids nor gas is formed from carbohydrates, nitrates are reduced, there is slight hydrolysis of starch, and milk is cleared without the precipitation of casein. Nakata (1927 a) claims that among the 160 isolants which he studied, there are two groups, one of which produces iridescent, black colonies on agar and liquefies gelatine, the other, opalescent white to brownish colonies, and does not liquefy gelatine. It loses its pathogenicity and virulence rapidly in culture unless it is transferred about every third day (Stanford and Wolf, 1917). They found that cultures four and a half months old are no longer viable. Nakata (1927 b) found the pathogen to remain alive about forty days on synthetic agar, but two hundred and seventy days in sterilized milk.

Life History.—The wilt-organisms grow and multiply within affected plants, and upon their death and disintegration are liberated in the soil. Here they survive throughout the winter, as shown by the fact that when plants are set in the same fields in the following season they become diseased. Field observations (Stevens and Sackett, 1903) indicate that the organisms are scattered by the washing of infested soils from higher land to lower, and by the conveyance of infested soil on tools, imple-

ments, the hoofs of work animals, and the feet of laborers. It is not known for what length of time the wilt bacterium can remain alive in the soil. In rotation experiments in which heavily infested soils were planted to crops not affected by wilt for three, four, five, or six years, it was found that 12.6 per cent of the plants were wilted after three years' cropping with immune plants, 18.9 per cent after four years', and 10.9 per cent after five years', and 2.3 per cent after six years'. The effectiveness of rotations depends somewhat upon seasonal conditions (Garner, Wolf, and Moss, 1917), but it appears that three years' cropping between successive crops of tobacco will be sufficient to bring wilt under control. Under no condition should tobacco be followed directly by a second crop of tobacco on wilt-infested lands, since such a procedure would undo the benefits derived from a rotation. The plots used in the above-mentioned rotations were contiguous, thus allowing ample opportunity for carriage of contamination from one to the other.

Observations generally bear out the statement that the introduction of the wilt organizm into new fields has followed the miscellaneous exchange of plants and their transportation from infested seed beds. Wilt became quite widespread before it was realized that the plants in the beds could be affected but not exhibit visible symptoms.

Influence of Environmental Factors.—The factor of most importance in favoring the development of wilt in the early portion of the growing season is wet weather. If this is coupled with high temperatures the progress of the disease is extremely rapid. If there has been a deficiency of precipitation throughout the entire growing season, and a drought prevails during harvest, a large proportion of the crop in infested areas of the flue-cured district will die. It appears that the roots near the surface of the soil are able to absorb sufficient water to keep the affected tobacco plants alive so long as the rainfall is normal. When, however, the soil near the surface becomes dry, since the larger, more deeply penetrating

roots are involved in decay, the plants quickly perish. Nakata (1927 c) found that the pathogen is viable in soil after fourteen months. It dies out rather quickly in soils when the water content corresponds with the "wilting coefficient." Sandy soils are apparently more likely to become heavily wilt-infested than clayey soils. Nematodes, moreover, are generally present in wilt-infested sections, and it seems that the injury which they bring about favors infection by the wilt germs. Injury to the root system, which cannot be obviated in transplanting and cultivating, also favors infection.

Bacteria Occurring in Tissues of Plants Affected with Granville Wilt.—It is quite apparent to anyone who has investigated Granville wilt or slime disease of tobacco that not all of the destruction of tissues of affected plants is due to *Bacterium solanacearum*. If one desires to isolate the pathogen in pure culture, he will be most likely to be successful if he severs the stem with aseptic precautions, considerably above the ground level, crushes out the slime from the xylem vessels, and then uses this slime as inoculum for dilution plates or to streak over the surface of plates of hardened agar. In the basal portions of the stem secondary invaders are practically always present and may interfere with the isolation of the pathogen. Honing[1] made a study of the kinds of organisms present in affected plants along with the causal organisms, and found a rather large variety. Among the more or less well-known ones that he found are *Micrococcus luteus* (Schroeter) Cohn, and *M. pyogenes* (Rosenbach) Migula.

Among others he listed and described 11 new species of bacteria as follows: *Bacterium langkatense, Bact. medanense, Bact. patelliforme, Bact. rangiferinum, Bact. Shuffneri, Bact. stalactitigens, Bact. sumatranum, Bact. zimnioides, Bact. zimnioides non-liquefaciens,* and *Corynebacterium piriforme.*

[1] Honing, J. A., Over Rottingsbacterien uit slijmziekte Tabak en djatte enkele andere van slijmziekte verdachte Planten (Bacterial species obtained from tobacco and other plants showing gummosis). Deli Proefstat. Medan (Sumatra). Jaargang 7, 6e afl., 223-253. 1912.

In addition to certain ones of these or all of these, one should expect to find, in decaying tobacco plants, any of the normal soil bacterial flora, a wide variety of different species.

Control.—Experiments on the control of tobacco wilt in North Carolina were conducted during the period from 1904 to 1917 and were directed along three lines as follows: (1) the search for wilt resistant varieties, (2) the treatment of the soil with various chemicals, and (3) the employment of various systems of crop rotation. The studies on varietal resistance, as previously mentioned, show that none of the species and varieties of *Nicotiana* and of its hybrids, either domestic or foreign, is sufficiently resistant to meet practical requirements in wet seasons. This is in accord with Honing's (1914) findings in Sumatra.

In the studies on the second phase of the problem various fertilizer treatments in which nitrogen, phosphoric acid, and potash were combined in varying amounts and from different sources were tried without success. Various acids and alkalis including sulphuric acid, nitric acid, acid potassium sulphate, quicklime, calcium carbonate, sodium carbonate, potassium carbonate, and sodium silicate were employed; but these materials render the soil unfit for the proper growth of tobacco when sufficient quantities are supplied to check the wilt organism. Among the germicides, fungicides, and disinfectants which were used without benefit were formaldehyde, mercuric chloride, potassium permanganate, chloride of lime, coal tar, pine tar, naphthol, lime sulphur, atomic sulphur, and Bordeaux mixture (Garner, Wolf, and Moss, 1917). It does not appear that any change of the chemical or physical properties of the soil could be expected to be effective. Nakata (1927 c) found that *Bacterium solanacearum* grows in soils with a reaction between pH. 6.0 to 8.1, and that this range of tolerance checks with that in culture media.

The work on control by means of crop rotation has demonstrated that this furnishes a satisfactory and prac-

tical method. Such crops as cotton, corn, oats, rye, wheat, clover, vetch, cowpeas, grasses, and sweet potatoes are not affected by the tobacco wilt germ, and may be grown in a rotation system. Various cropping systems have been suggested by Moss and Wolf (1917) for sections which are wilt infested. Some of these are being employed with success in North Carolina.

The early field tests on rotations were not effective because they included the practice of leaving the fields fallow for a period of "rest" or "lying out." When this was done, such common weeds as ragweed, fleabane, horse nettle, croton, Jimson weed, and *Eclipta alba*, all of which are suscepts, throve in abundance. These weeds make it difficult to starve out the wilt organism by crop rotation.

Added precautionary measures which will help to prevent the introduction and spread of wilt germs include the following:

(1) Seed beds should be thoroughly burned or steamed, and they should be situated so as to prevent drainage waters from infested fields from reaching them.

(2) Plants from an infested bed may be the means of introducing wilt into a new field or into a new locality.

(3) Soil clinging to farm implements or to the hoofs of farm animals allowed to roam at will may be a means of introducing wilt to new situations. These findings from experimentation on control in North Carolina are quite in accord with those from the Dutch East Indies and Japan.

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Wildfire of Tobacco

The name "wild fire" was applied by growers, because of the apparent suddenness of its appearance and rapidity of its spread, to a peculiar leaf spot disease of tobacco that was first definitely recognized in North Carolina in 1917. It has subsequently spread to practically all parts of the world where tobacco is grown except to Australia and the Dutch East Indies. Many observations and experiments dealing with wildfire have been published that are in accord in all essential features, so that it is to be regarded as one of the best known among the diseases of tobacco of bacterial origin.

Suscepts.—The question of whether cultivated plants closely related to tobacco, and weeds growing near diseased tobacco plants, might be attacked was given attention early in the investigations. Wolf and Foster (1918) noted small yellowish spots with necrotic centers on cowpeas *(Vigna sinensis)* planted between the hills of a badly diseased crop of tobacco. They isolated an organism from these spots that produced typical wildfire when sprinkled on tobacco, but only a few lesions appeared on cowpeas similarly inoculated. These lesions appeared to have originated at the locus of punctures made by leafhoppers so that the organism was regarded as a semiparasite on cowpea. They were unsuccessful in their attempts to produce infection in peppers *(Capsicum annuum)*, and Jimson weed *(Datura stramonium)*. Later, Wolf (1922) was unsuccessful in securing infection in Irish potato *(Solanum tuberosum)*, tomato *(Lycopersicum esculentum)*, eggplant *(Solanum melongena)*, and horse nettle *(Solanum carolinense)*. Chapman and Anderson (1921) noted infected tomato plants growing in seed beds among tobacco seedlings, and as a result of inoculation of eggplant, *Petunia* sp., and pokeweed *(Phytolacca decandra)* infections were secured. Clinton and McCormick (1922) reported doubtful results from inoculation of eggplant and pepper, but were unsuccessful in producing infection in pokeweed, tomato, and Jimson weed.

Johnson, Slagg, and Murwin (1924) secured infections on plants in 24 phanerogamic families. They em-

ployed virulent cultures and inoculated without wounding and also with needle punctures, so that a droplet of a watery suspension of the pathogen was left at each needle puncture. The following species of *Nicotiana* became infected: *N. alata grandiflora, N. Bigelovii, N. glutinosa, N. langsdorffii, N. paniculata, N. repanda, N. suaveolens N. sylvestris, N. angustifolia, N. atropurpureum, N. calyciflora, N. campanulata, N. chinensis, N. laterrima, N. macrophylla,* and *N. trigonophylla.* Among other solanaceous species upon which infection occurred were peppers, eggplant, tomato, potato, Jimson weed, nightshade *(Solanum nigrum),* and ground cherry *(Physalis grandiflora).*

Anderson (1925) found that there was no significant degree of resistance to wildfire in any of the 41 agronomic varieties of *Nicotiana tabacum* which he tested. He tested, in addition, many species of *Nicotiana* with results that accord with those of Johnson *et al.* (1924). Among the susceptible species additional to Johnson *et al.'s* list, which he tried, are *N. colosea, N. plumbaginifolia, N. quadrivalvis, N. sanderae, N. longiflora,* and *N. wigandioides.* He regards *N. rustica, N. alata, N. repanda, N. attenuata* and *N. nudicaulis* as highly resistant. Böning (1930) observed that a yellow-flowered *N. rustica* remained practically free from attack.

The inoculation studies of Johnson *et al.* (1924) resulted in infection upon such well-known crop plants as muskmelon, watermelon, pumpkin, cucumber, bean, pea, peanut, soybean, cowpea, vetch, clovers, alfalfa, sweet clover, wheat, rye, oats, barley, timothy, cabbage, turnip, radish, rape, mustard, lettuce, buckwheat, beet, spinach, cotton and carrot, and upon a large number of weeds and ornamental flowering plants. Clayton (1934), however, regards only species of *Nicotiana* as true host plants for the wildfire organism.

The Disease

Names.—The name "wild fire," first definitely employed in 1917, appears to have been almost universally

adopted wherever this disease has made its appearance. In Germany this name becomes *wildfeuerkrankheit* or *brennfleckenkrankheit* and in France *feu savage*. In the Union of South Africa growers applied the name *verterende roest* (literally consuming rust) to this disease at the time of its first appearance in 1921.

History and Range.—The origin of wildfire, as is the case with many diseases, is unknown. It is known to have been seen in North Carolina in 1916 and no doubt existed prior to that time. In the following year it was collected from practically every tobacco-growing county in North Carolina. In 1919 it appeared in the Connecticut Valley and in 1920 in the Burley and dark tobacco-growing sections of Kentucky and Tennessee. It also appeared about this same time in Virginia, Georgia, Florida, Louisiana, Pennsylvania, Ohio, Wisconsin, and Maryland. It was first reported from Pretoria, South Africa, in 1921, and in the same year from the Piet-Retief district there. The next season it appeared in the Pietersburg and Rustenburg districts of South Africa. Since then it has been reported from Canada, Argentina, Hungary, Bulgaria, Rumania, Belgium, France, Germany, Russia, and the Philippines. Pateff (1928) states that it probably was present in Bulgaria in 1913.

Importance.—Wildfire is the type of disease which is sporadic in its appearance and in its severity. In 1917 it was universally conceded by growers in North Carolina that this disease is the most destructive one to which tobacco is subject (Wolf, 1922). The losses that year ranged from those which were inappreciable to the loss of practically the entire crop on many farms. The next season the losses were about equally severe, but in 1919 they were of minor importance in North Carolina. Clinton and McCormick (1922) observed wildfire in 67 of the 125 fields that they inspected in Connecticut. Estimated damage in these fields ranged from none up to a total loss, with a number of fields in which the damage was 60 to 70 per cent.

In 1920 wildfire occurred in about 70 per cent of all

the fields of tobacco in Virginia and occasioned very heavy losses. Chapman and Anderson (1921) estimated that it occurred in about one-fifth of all the seed beds in Massachusetts, and caused serious losses in the fields. The next year it occurred in about half of the seed beds and fields in the Connecticut Valley but was less disastrous than in 1921.

Since 1919 it has been localized at widely separated points in the flue-cured-tobacco district, and has not involved any extensive areas.

Appearance of the Disease.—Wildfire is essentially a leaf-spot disease, although it has been found upon the flowers and seed pods. It may appear upon the foliage in any stage of the plant from the time the seed leaves appear to maturity. The symptoms of the malady upon the foliage are prominent, and differ from all other leaf spots sufficiently to be easily recognized by the ordinary observer. Weather conditions and age of the affected leaf greatly modify the appearance of the disease.

In North Carolina, wildfire makes its appearance in the seed beds during the last week in April or the first week in May. Upon the smallest plants which have been dwarfed or retarded by crowding or have come from seed which were delayed in germinating, there appears what may be called a wet-rot stage. The leaf margins and tips of such plants are involved in a rapidly advancing wet rot with a water-soaked zone between the living and the dead tissues. Often the entire leaf is rotted away, or the infected tissues may dry up and fall away, leaving the mutilated leaf apparently healthy. The bud-leaves of such plants are pale, erect, and slow in developing. Such plants may perish in the seed bed or may not survive transplanting. This stage, which may properly be regarded as the first, involves plants in patches, and does not occur throughout the bed. The diseased areas are usually on the lower sides of the beds, in the dampest situations. This wet-rot stage has frequently been observed in North Carolina in beds in which larger plants showed none of these symptoms. Chapman

Fig. 62. Young lesions of wildfire on the leaf of a tobacco seedling. The yellow halos are characteristic. The white spots are perforations made by flea-beetles. Sometimes halos surround the perforations made by the feeding of flea-beetles indicating that they may transfer the wildfire bacteria.

Fig. 63. Mature lesions of wildfire in which the entire leaf has become yellow. The centers of the lesions are light brown, and the borders are dark and appear to be water-soaked.

Fig. 64. Flue-cured tobacco leaf rendered worthless by the use of wildfire disease. In rainy seasons the lesions fuse and the tissues decay and fall away.

Fig. 65. Wildfire lesions on seed pods. Seed from such pods are infected or contaminated and, when sown, will produce affected seedlings.

and Anderson (1921) and Clinton and McCormick (1922) have described this as a very early stage on seedlings, and it has been illustrated by the latter in their Plate XXIX, Figure f.

On the larger plants, which have approached the size for transplanting, the first evidence of disease is the appearance of circular, yellowish green areas, about one-sixth to one-fifth inch in diameter. Within the next twenty-four hours, a minute, dead, brown speck, about the size of a pinhead, appears in the center of the spot, and the wide, yellowish green border, or halo, has become more prominent. This stage is followed within the next few days by an increase in size of both the central, dead area and the surrounding halo, forming a spot one-half inch or more in diameter, with a border of water-soaked appearance, which marks the margin of the central, dead, brown part. When the spots are numerous, they fuse, making large, irregular, dead areas. Not uncommonly, too, one-half of the leaf may be more seriously affected than the other, and, in consequence, such leaves become twisted and distorted. This third stage is not prominent in the seed bed, but shows to best advantage during June and July on plants in the fields.

The width and prominence of the water-soaked band depend upon moisture conditions. In dry weather the diseased areas are tan-colored to dark brown, with the lightest color at the center, and a narrow dark band at the border. During such weather these dead areas remain intact. When dewy nights and intermittent showers occur, however, the entire spot is soft and water-soaked in appearance, and the dead areas rot out so that the leaves present a ragged, torn appearance. This may properly be regarded as a fourth stage.

The most constant and dependable character for use in a field diagnosis of wildfire is the yellow halo, which persists in all stages of the disease.

Wildfire lesions on seed pods appear as rather prominent brown spots. This form of the disease has been collected in several localities, but is not particularly char-

acteristic, since other agencies cause the formation of quite similar spots on pods. The lesions on flower parts are small, brown, and irregular, and have not been seen except in artificial inoculations. Chapman and Anderson (1923) noted light brown, sunken wildfire lesions on the stalks of tobacco plants in Massachusetts.

The wildfire organism is among a large group of plant pathogens whose members are known to invade parenchyma tissues. It is confined largely to the intercellular spaces (Wolf and Foster, 1917; Clinton and McCormick, 1922; and Chapman and Anderson, 1921), at least until the cells have collapsed. Hill (1930) has shown that the bacteria multiply within the intercellular spaces to the extent of forming a mass of organisms that are imbedded within a gelatinous matrix of zoogloea. This zoogloeal mass slowly nigrates between the cells and thus extends the area of invasion. Other bacterial forms are always present in mature lesions and no doubt aid in the disintegration of the tissues. Johnson and Murwin (1925) were able to demonstrate the production of toxin in cultures of the wildfire organism. They suspended potato agar cultures of the pathogen in water, filtered the suspension through a Berkefeld, tested the filtrate to establish its sterility, and then used this filtrate as inoculum. Typical yellow halos developed on the inoculated tobacco plants within twenty-four to forty-eight hours. Platings from these yellow spots, moreover, showed that the tissues were sterile. When the pathogen was repeatedly washed to remove this toxin, and the washed organisms were used as inoculum, it required several days longer to produce typical lesions than when the toxin alone was used as inoculum. This evidence must be interpreted to show that a potent exotoxin is excreted. This toxin is apparently not specific for tobacco plants, which fact aids in explaining the production of lesions on such a wide variety of plants, following inoculation with the wildfire organism. This explanation is in accord with the recent findings of Clayton (1934).

Cause.—The cause of tobacco wildfire is a bacterial organism, *Bacterium tabacum* Wolf et Foster (1918). Many growers still believe that it is caused by such factors as rainy weather, improper fertilization, or insect injuries. These several factors are known to influence the progress of the disease and dissemination of the pathogen, but unless the specific germ or organism is present, wildfire will not appear. This has been repeatedly demonstrated by carefully conducted experiments in North Carolina, and has been confirmed by investigations in Connecticut, Massachusetts, Wisconsin, New York, and in many of the foreign countries where wildfire has been studied. Chapman and Anderson (1923) state: "In Massachusetts the writers have made numerous isolations from all types of lesions described above and have invariably obtained pure cultures of an organism that has never failed to produce the typical disease when healthy plants were inoculated with these pure cultures."

The wildfire organism is a non-spore-forming rod, 1.4 - 2.8 x .5 - .75 μ, and is motile by means of 1 to 6 polar flagella. It is aerobic, Gram negative, does not hydrolyze starch, reduce nitrates, nor form gas from carbohydrates. Colonies on agar are grayish white, circular, smooth, glistening, and entire margined. Gelatine is liquefied by it, casein precipitated from milk, and acids formed from dextrose, sucrose, mannit, and galactose.

Hibernation of the Wildfire Organism.—Observations, generally, are in accord in showing that wildfire invariably begins in the seed bed and is introduced into the field at time of transplanting. The investigations that have sought to discover the means of its introduction into the seed beds show that there are at least five possible sources of inoculum: (a) diseased seed, (2) cured leaves of tobacco, (c) infested soil, (d) contaminated seed-bed canvas or covers, and (e) fertilizers.

Seed.—The best evidence which has yet been secured that the wildfire organism is seed-borne comes from finding that the seed pods are subject to attack, thus making entirely possible the contamination of the seed within the

pods. It seems probable also that seed might become contaminated during harvesting and cleaning, if the foliage of the seed-plants were diseased. Further evidence that the organism may be seed-borne was secured by contaminating seed with pure cultures of the wildfire germ, and then sowing these seed in experimental seed beds at the Tobacco Experiment Station, Oxford, N. C. When these beds were examined on May 7, all plants in the two compartments sown with contaminated seed during the preceding February were seriously infected. No evidence of wildfire was found on plants in any of the adjoining compartments, and none developed subsequently.

Johnson and Murwin's (1925) experiments show that the wildfire germ can remain dormant on the surface of seed for as long as two years. They do not regard seed as a common source of inoculum for seedlings in the seed bed, but advise against sowing seed saved from diseased plants, unless such seed have been adequately disinfected. Clinton and McCormick (1922) are of the opinion that seed is probably one of the sources of inoculum, and cite the case of a grower who, in 1920, saved seed from diseased plants. Wildfire appeared the following spring in his seed beds, and also in those of three other growers to whom he had given some of this seed.

Cured Tobacco.—There is evidence that the wildfire organism may be introduced into the seed beds through spitting on the seedlings when workmen weed or "pick" the seed beds or when they draw plants preparatory to setting them in the fields. This is more likely to occur when they chew "natural-leaf," air-cured tobacco than when they use plug tobacco or snuff. Attempts to isolate the germ from air-cured diseased leaves, eight to nine months old, have been unsuccessful (Wolf, 1922), as have those in which flue-cured leaves have been used. Clinton and McCormick (1922) succeeded, however, in inoculating tobacco by the use of diseased leaves that had dried for 198 to 298 days. Johnson and Murwin (1925) found that the wildfire bacteria readily overwinter on cured leaves that are kept dry during the interval between grow-

ing seasons. Chapman and Anderson (1922) were unable to secure infection from diseased leaves that were left lying on the ground all winter.

Fertilizers.—The possibility of the introduction of wildfire germs into seed beds and fields through commercial fertilizer materials is remote. Before tobacco stems are incorporated with fertilizer materials they are processed, which treatment should insure their complete sterilization.

Infested Soil.—Because the isolation of plant-pathogenic bacteria from soil is such an extremely difficult task, it has been impossible to prove absolutely that soil from fields or seed beds that have grown diseased plants, is a source of inoculum for wildfire. The evidence which has been secured along this line, however, indicates that account must be taken of soil as a source of inoculum, especially in seed beds (Wolf, 1922). In the experiments on soil infestation, old seed beds were used which had borne diseased plants during the previous year, and which were not fired prior to planting. Seed from a locality where wildfire was absent were sown in these beds, and new cloth was used as covers. The disease developed in several of these beds, but not in all.

In another test, seed were planted in flats in the greenhouse in soil from a field in which the crop was practically a total loss. The seedlings in these flats showed no evidence of wildfire, due, perhaps, to the lack of suitable moisture conditions. The attempts to prove the presence of the organism in this soil by sprinkling a suspension on healthy plants were also unsuccessful. Clinton and McCormick (1922), however, succeeded in one trial in infecting tobacco plants from the use of infested soil. Johnson and Murwin (1925) concluded that the wildfire bacteria do not overwinter in moist soil in Wisconsin and that there is no danger in using land for tobacco on which the previous crop was infected, provided the stubble and refuse from the previous crop had been thoroughly plowed under.

Contaminated Cloths.—No positive proof has been secured that cloths from infested seed beds are a source of inoculum for beds in the succeeding year. It is conceivable, though, that old cloths might harbor wildfire germs, when it is recalled that the cloths are not removed until a few days before time for transplanting, at which time they are usually rolled up and put under shelter. It has been impossible to isolate wildfire bacteria from used cloths, but indirect proof that such cloths harbor the parasite is shown by the following experiments (Wolf, 1922). New seed beds, thoroughly fired, and so located with reference to distance and to surface drainage that there was no chance of contamination from neighboring fields, have been used in these tests. When seed of known healthy origin were used in certain of these beds, and the beds were covered with new cloths, the plants remained free from wildfire. When, however, other beds were planted with seed from the same source and covered with cloths taken from beds which had been affected with wildfire during the previous year, the disease appeared.

Fromme (1921) cites the following incidents to show inoculation from the use of old cloths in Virginia:

> The germs of angular leaf spot and wildfire may be carried on old canvas and cause infection in the plant-bed. This was proved by experiments, and also by the following cases: R. H. Mantiply, of Amherst County, used seed which had been treated but did not boil the canvas. Tilden Gooch used some of the same seed and boiled his canvas. Wildfire and angular leaf spot were found in Mr. Mantiply's bed, but not a trace of either could be found in the bed of Mr. Gooch.

Factors Influencing the Severity and Spread of Wildfire.—Both nutritional and weather conditions have been observed to exert a controlling influence on the severity of wildfire once it is present in the field. Experienced growers have learned that the excessive use of nitrogen, especially nitrate of soda, produces a rapid, tender, watery type of growth. The same type of growth results from proximity to ditches or to situations which are

normally well supplied with water and organic matter. All observations, furthermore, are in accord in showing that wildfire is much more destructive to such plants than to those which have made a normal growth. It has also been learned that the use of an adequate supply of potash produces plants which are hardy and less liable to succumb to leaf spot diseases.

All observations, wherever wildfire has been investigated, are in accord in showing that moisture is of primary importance in influencing both the severity and spread of wildfire. Whenever a rainy period of several days' duration occurs, it may be followed by an outbreak of the disease. Few or no new infections occur during a dry period.

If rain is accompanied by high wind, ideal conditions are provided for the rapid spread of wildfire, since the causal organisms are driven into the leaf tissues and the water-soaked areas are rapidly transformed into lesions. A considerable number of cases recounted to the writer by growers are, in general, like the following observation (Wolf, 1922). In one locality, near Eagle Rock, N. C., no disease occurred except in the case of a field of approximately an acre of newly cleared land. The plants used in setting this field were unknowingly procured from a seed bed that contained wildfire-affected plants. When this field was again observed, about a week after a rain storm, which occurred about the middle of July, 1917, a field in the direction in which the wind blew was seriously diseased. The infection was much more abundant near the diseased field, and gradually diminished in severity in the direction away from the diseased field. It was very obvious here that the inoculum was spread by wind-blown rain. Wind, alone, however, during dry periods does not spread the inoculum. Flea beetles (*Epitrix parvula* Fab.) are able to disseminate the wildfire organism, as shown by its isolation in pure cultures from the bodies of flea beetles, and successful inoculation of tobacco (Wolf, 1922). This conclusion is supported indirectly by the frequency with which lesions appear around perforations

made by the insects. Clinton and McCormick (1922) also noted that lesions arise where flea beetles have eaten holes through the leaves.

Control

In Seed Beds.—The fact of greatest importance which has thus far been established in regard to wildfire and which must be made the basis for all remedial measures is that the disease has its origin in the seed bed. Once the disease has been established in the field, there is little that can be done to check its spread. One can avoid introducing it into the fields at time of transplanting by the use of healthy plants. This can be accomplished by the following procedure:

1. Use seed of known healthy origin, which are known to have come from a wildfire-free field, and which have been protected at all times from subsequent contamination. If such seed cannot be procured, then place the seed in a cheesecloth bag in a jar, or pour them into a jar and cover the top with a cheesecloth. The seed should then be soaked for ten minutes in a formaldehyde solution. This solution should be of the strength of one tablespoonful of formaldehyde to one pint of water. It is necessary to observe two precautions or injury to germination will result. The seed should not be treated over ten minutes, and they should immediately be thoroughly washed in several changes of water. They are then ready to be spread out to dry. It is preferable to treat the seed a few hours before sowing. Silver nitrate (1:1000) has been found by Johnson and Murwin (1925) to be the most satisfactory disinfectant.

2. If wildfire was present in the beds the previous year, either make beds on new land away from all possible contamination by drainage from infested beds or fields, or thoroughly burn the old beds. If wildfire is absent, and one wishes to use the same seed bed for several years, it is well, as a precautionary measure, to pull up all plants left as soon as the transplanting season is over and to cover the bed deeply with pine straw, so as to

guard against seed-bed diseases, to keep down all weed growth, and to conserve fertility.

3. Use new covers, or if old ones are employed, they should be sterilized by heating in boiling water. This precaution is necessary, since it has been found that old covers may be a source of inoculum.

4. Avoid inoculating your own beds by not visiting the infested beds of neighbors.

5. As a final precaution, the seed beds may be sprayed or dusted with Bordeaux mixture. If commercial preparations are used, the package contains directions for use. If home-made Bordeaux mixture is employed, it should consist of one pound of bluestone dissolved in a gallon of water, and one pound of quicklime slaked in a gallon of water. Each should then be diluted to make 5 gallons, and the two should be poured together to make 10 gallons of spray. This quantity should be sufficient to cover the seedlings occupying about 600 square feet, if properly applied. Begin spraying about four or five weeks before transplanting, and spray each week or ten days so as to keep the new growth protected with a coat of spray. Excellent control of wildfire in seed beds has been accomplished in Connecticut and Massachusetts by spraying and dusting. Bordeaux mixture, in some flue-cured areas, is injurious to tobacco seedlings, especially when several applications must be made.

In Fields.—

1. Use only plants known to come from disease-free seed beds in setting out the fields. Do not buy or exchange plants unless they are known to be free from wildfire. If it is impossible to get healthy plants, and the beds are only slightly diseased, use only those which are apparently free. Inspect the fields about a week afterward, remove the diseased plants, and destroy them. Reset with healthy ones. After the plants have started to grow in the field, make a second inspection and remove the lower affected leaves. The elimination of infective material may effectively decrease the injury to the crop which might occur in late summer.

2. Fields which have grown a diseased crop during the previous year should not be planted with tobacco. The organism may live over in the field and may infect the next crop when tobacco follows tobacco. If it is necessary for tobacco to follow tobacco upon infested fields, there appears to be little probability that the disease will become serious when healthy plants are set out. Fields in which the crop was badly diseased have been set with healthy plants in the following season, and a healthy crop was grown. It is well, however, aside from the danger of wildfire, to employ a system of crop rotation.

3. Growers have frequently asked whether the disease in the field can be checked by spraying. The few experiments that have been made along this line have not given very satisfactory results. Even if it could be accomplished, such a procedure cannot be recommended as a field practice, because of the expense involved and the possible injury to the market value of the crop.

4. Field observations indicate that low-topping is to be avoided. Low-topped plants suffer greater injury by wildfire than plants with the normal number of leaves, especially when rainy weather occurs as the crop is maturing.

5. The removal or priming of affected leaves is of doubtful value on a practically mature crop. In some cases it appears to have retarded the spread of wildfire, whereas in others no good has been accomplished by priming.

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ANGULAR LEAF SPOT OF TOBACCO

The disease of tobacco to which the name "angular leaf spot" is applied is one of those troubles which occur year after year, but becomes seriously destructive only in occasional seasons.

SUSCEPTS.—This disease appears to be restricted to *Nicotiana*. It has been observed upon all of the common varieties of cultivated tobacco grown in Virginia, and none of the 26 varieties tested exhibited any marked degree of resistance. At least none was sufficiently resistant to indicate that control might be accomplished through choice of varieties (Fromme and Wingard, 1927). The disease in Kentucky on seedlings of dark-fired types, even when grown in the same seed bed with severely infected White Burley tobacco, are only very slightly infected (Valleau, 1932).

HISTORY AND RANGE OF THE DISEASE.—The study of angular leaf spot dates from 1917, although it very probably existed in Virginia for a considerable number of years prior to 1917. Fromme and Wingard (1922) suggest that it is possibly native to the American continent and that it perhaps antedates the early culture of tobacco

by the colonists. Excerpts from early letters that mention "firing," "rust," "field fire," etc., which they quote, may refer to this disease.

A survey made within Virginia showed that angular leaf spot was of very general distribution throughout the state by 1920. It has been reported also in North Carolina, South Carolina, Georgia, Kentucky, Tennessee, Louisiana, and Indiana, and probably is identical with a leaf spot disease that occurs in Maryland, Ohio, and Pennsylvania. As will be noted later, the pathogen is seed-borne and doubtless has been introduced into many other tobacco-growing sections along with the seed. This is known to be the case in Rhodesia, and other parts of South Africa, Bulgaria, Germany, Nyasaland, Canada, and the Philippine Islands.

Economic Importance.—In certain years, including the seasons of 1917, 1919, and 1920, this disease appears in epiphytotic form. It is productive of losses every year, but in some years the losses are serious in restricted areas. Under unusual conditions, as was noted in 1933, large brown to black angular spots appear on the seedlings while they are still in the seed bed. Symptoms like this are unusual except on plants that have become established following transplanting. In 1920, 85 per cent of the fields inspected in Virginia contained diseased plants, and the estimated loss for the state was 21,853,000 pounds. Undoubtedly proportional losses occurred in the remainder of the flue-cured-tobacco section. The disease not only reduces the grade of the cured product but also the yield.

Symptoms.—Angular leaf spot may appear on plants in any stage of development from seedlings to mature plants. Surveys of seed beds, made in 1919-1921 inclusive, showed its presence, in Virginia, in 82 of the 142 beds inspected. It is most likely to be found in those parts of the beds in which the plants have made most vigorous growth, especially as the plants approach the size for transplanting. As a rule, two or three angular to irregular, black to very dark brown spots appear on a

single leaf. These spots are much smaller than those on mature leaves, and are characterized by a narrow, translucent, marginal zone. If the spots are numerous or occur on the leaf margin, the leaf becomes distorted. The necrotic, central portions become broken and fall away.

On plants in the field, as described by Fromme and Murray (1919), the spots are characteristically angular and rarely exceed 1 cm. in diameter. The spots on dark tobacco may become zonate and be considerably larger. Young spots are very dark, almost black, and as they become older and desiccated they become dark brown to tan. The leaf tissue bordering these spots is yellowish, which decreases in intensity into the normal green, and there is never a yellowish halo as in wildfire. The centers may fall out, and in very severe infection little besides the veins remains. If the leaves are more severely affected on one side of the midrib, they become drawn and distorted from growth tensions.

In cases where angular leaf spot is prevalent, flower infections may be noted on plants permitted to set seed. The calyx lobes are blackened and recurved. The spots on corolla lobes and capsules are not easily distinguished from those produced by the wildfire organism. They are paler, larger, and lack the conspicuous halo.

Cause.—Angular leaf spot is a bacterial disease, although a variety of beliefs have been held by growers as to the cause. They have regarded it as due to excessive rains, and deficiencies of fertilizer elements or excessive amounts of certain of them. Fromme and Murray (1919) first showed that it is caused by an organism which they called *Bacterium angulatum*. This has subsequently been confirmed by other investigations within the United States and abroad.

The bacterium is a non-spore forming, aerobic, Gram negative, rod-shaped, organism, 0.5 by 2.0 - 2.5 μ, motile by means of a 3 to 6 polar flagella. Agar colonies are convex, smooth, glistening, center dull white and opaque but with a clear undulate margin. On potato cylinders it forms a dull yellow pigment. It is capable of liquefying

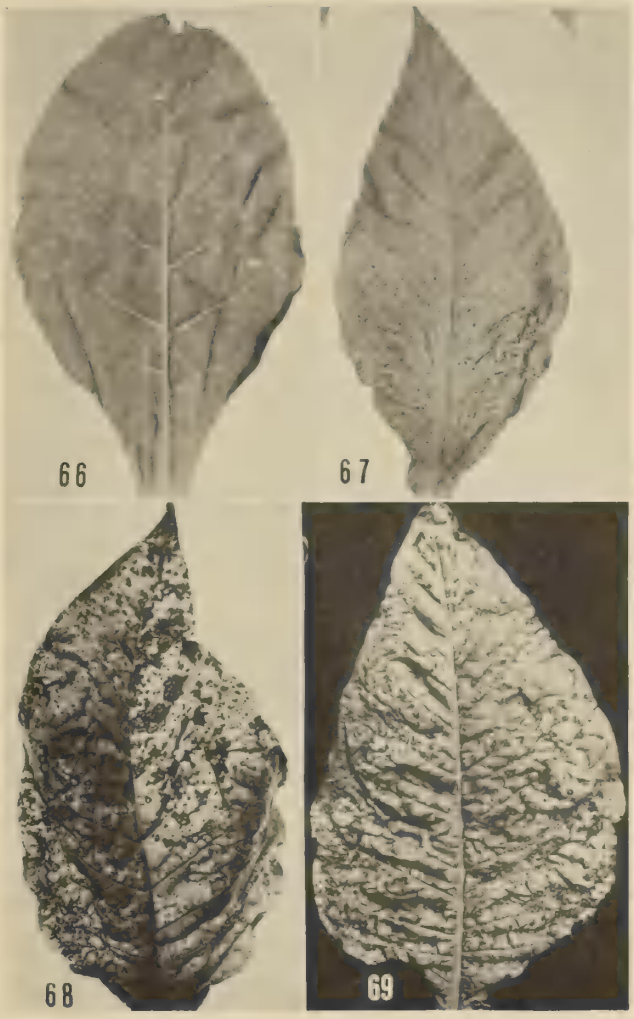

FIG. 66. Tiny, dark lesions of angular leaf spot on the leaf of a seedling.

FIG. 67. Numerous, irregular, dark brown to black, sharply angular spots on fully grown leaves, characteristic of the angular leaf spot disease.

FIG. 68. Mature leaf severely affected with angular leaf spot.

FIG. 69. Leaf that has been rendered worthless by angular leaf spot.

gelatine, of forming acid from dextrose and sucrose but not from other carbohydrates, and of clearing milk without the coagulation of the casein. It does not form gas nor reduce nitrates.

Pathogenicity.—Proof of pathogenicity has been established by inoculation of plants in the greenhouse (Fromme and Murray, 1919). Pure cultures were atomized upon 150 seedlings plants, and 96 per cent became infected. Under conditions of high humidity and high temperature, the stage of incubation requires four to five days. This period is extended with unfavorable environmental conditions. Infections readily follow the application of the inoculum with a cotton swab, or when the leaves are gently rubbed between the thumb and fingers.

Dissemination.—The studies on this point by Fromme and his associates show that the introduction of angular leaf spot into seed beds and thence into the field is resultant from several sources which they summarize as follows:

1. The organism is seed-borne as shown by planting tests of naturally and artificially contaminated seed and by seed disinfection tests.

2. The pathogen is carried over from one year to the next on chaff, leaves, and trashy parts of the seed heads.

3. It may survive the winter on seed-bed covers or canvas.

4. Evidence indicates that the pathogen may be introduced into the seed bed with manure or with tobacco trash.

5. It may overwinter in the soil under certain conditions.

Early observations indicated a correlation between the occurrence of angular leaf spot in the seed bed and in the field, and suggested the possibility of its introduction into the beds with the seed.

In one test (Fromme and Wingard, 1927) 90 different lots of seed with corresponding checks of untreated seed were used. Formaldehyde (1:16) and corrosive sublimate (1:1000) were used on 45 lots each of the treated seed. Newly cleared land was selected, new lumber em-

ployed for the compartments, and new cloths to cover the beds. None of the disinfected seed developed plants affected with angular leaf spot. The disease developed, however, on twenty-three of the check lots.

In another of their tests on seed transmission, one lot of seed was contaminated with pure cultures, another with the cleanings from seed, and another lot was used as a check. Ten per cent of the plants in each of the first two became infected, and the check remained healthy. Infection resulted when overwintered, air-cured, diseased leaves were used as inoculum.

Cases have been observed in which it was clearly indicated that the organism was overwintered on canvas. In one instance, all sanitary precautions were taken to eliminate the bacterium from two beds except that an old canvas was used on one of them. The plants in this bed became diseased, while those in the one covered with a new canvas remained healthy.

When fields are in close proximity, inoculum may be transferred from one to the other through the agency of man, during such operations as topping, worming, suckering, and cultivation. Tobacco culture necessitates considerable handling of the plants, and this favors the spread of inoculum, especially if the plants are wet. No spread of inoculum occurred in two fields, separated by a roadway, although one field was infected early and the other remained free from angular leaf spot to maturity (Fromme and Wingard, 1922). In two fields of tobacco that were separated by a strip of corn 150 feet wide there was no spread from the infected crop in one field to the healthy crop in the other, even though both were cared for by the same person. Wind-blown rain, among natural agencies, appears to be of little consequence in field to field spread of this disorder. It is, of course, a prime factor of dissemination within any particular field.

Effect of Environmental Factors.—As has been stated, angular leaf spot may not appear in epiphytotic proportions in two successive years. Observations indicate that the factor of primary importance in favoring

the presence of the disease is rainfall. The correlation between heavy or prolonged rainy periods and the occurrence of angular leaf spot is so striking that growers consider rain as the primary cause. When diseased leaves are wetted, the bacteria swim out to the surface, and other drops of rain then splash these bacteria to other leaves on the same or on other plants. When tobacco approaches the topping stage, the close proximity of leaves makes conditions ideal for spread of the organism in this way. During the months of July and August, 1920, in Virginia, the rainfall was approximately 12 inches, an epiphytotic season, whereas in corresponding months of 1921 the precipitation was about 3 inches, a season in which there was no appreciable injury from angular leaf spot.

The belief that the disease is caused by fertilizers is founded on the fact that the susceptibility of the tobacco may be modified by nutrition. In experiments involving the use of various amounts of mineral elements, the lesions on the poorly nourished, slow-growing plants, when inoculated, remained small, and became large and abundant on thrifty rapidly growing plants.

Differences in severity of the disease may be noted to be related to topping practices. Plants that are topped to 8 leaves are more severely affected than those topped to 14 or more leaves.

Control.—Since it has been demonstrated that the use of infected seedlings is responsible for the presence of the disease in the field, control measures center about the problem of securing healthy seedlings. For this reason it is suggested that the seed should be selected from pods that are free from infection. Covering the flower stalks with paper bags is an aid to the production of noninfected seed. Although seed selection should be expected to be helpful, it should be supplemented by seed disinfection. This may be accomplished by soaking the seed for ten minutes in 1:1000 solution of corrosive sublimate, then rinsing them thoroughly in water, and spreading them to dry. Formaldehyde 1:16 is equally effective. Fromme and Wingard (1922) treated 2,500

lots of seed, 900 with formaldehyde and 1,600 with corrosive sublimate. The following tabulation shows the results obtained in 70 beds sowed with treated seed as compared with 26 beds sowed with untreated seed. These beds were divided into three classes as follows: Class A. Treated seed used and all sanitary precautions practiced. Class B. Treated seed used and all other sanitary measures omitted. Class C. Seed not treated.

Class	Total number of beds	Number of beds with angular leaf spot		
		None	*Trace*	*Moderately severe*
A	54	30	11	13
B	16	0	4	12
C	26	2	2	22

These tests show that seed treatment is beneficial but that it must be supplemented to get practical control.

It might be anticipated that the use of old seed would be of advantage since with aging the angular leaf spot organism should perish. No one has determined how long the germs will survive on the seed, but seed that are two or more years old should be free from contamination.

The seed beds should be placed each year on new ground, and be well removed from fields in which tobacco has recently been grown. Manure containing refuse from a recent crop should not be used on beds. Poles or boards that have been used previously should not be used in constructing the new beds. While no clear-cut cases have been found of a carry-over of inoculum in old beds or in fields, yet it appears highly probable that the soils could become contaminated. Unplowed stubbles, in mild winters, remain alive and might serve to keep the pathogen alive. In sections where cropping with tobacco in successive years is practiced, there is no evidence of the survival of the organism on the old plants or in the soil (Fromme and Wingard, 1922).

New cloth or canvas should be employed each year, or if the old one must be used, it should be boiled to disinfect it. Evidence from observation of seed beds indicates that

the causal organism can remain viable on old canvas from one season to the next, but no one has actually isolated the organism from old canvas.

The inoculum may be distributed on the hands and clothing of the worker during such operations as pulling plants and setting them in the fields. This can be avoided at the time of transplanting. The exchange of plants and exchange of labor may be the means by which the plants become contaminated.

The plants should not be worked or handled while they are wet, since infection necessitates the presence of a film of moisture to permit entrance of the organism into the pores of the leaf.

It is advisable to practice rotation of crops to diminish the possibility of carry-over in the soil. If tobacco is to follow tobacco, the stubbles should be turned under as soon as possible after harvest.

Valleau (1923) considered chewing tobacco the principal source of contamination of seed beds in Kentucky, and recommended the avoidance of the use of chewing tobacco while preparing, sowing, inspecting, weeding, or transplanting.

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Wisconsin Bacterial Leaf Spot

Several distinct leaf spots of bacterial origin are now known to occur on tobacco. Some of these can be definitely recognized in the field because of characteristic symptoms, while others, unless subjected to special study, may easily be confused with several other disorders of parasitic or non-parasitic origin. The disease under con-

sideration is of the latter type and probably has been present in Wisconsin for over fifty years.

Suscepts.—Johnson (1923), who first recognized this disease and described the causal organism, noted it on several varieties of cultivated tobacco. In addition, he secured infections, following inoculation, upon *Nicotiana glauca* and *N. rustica*, and upon tomato. The only extensive studies of suscept range are those of Săvulescu and Rădulescu (1929) in Rumania. They found that the same organism occurs naturally on Jimson weed, *Datura stramonium*, and several other weeds. They were successful by artificial inoculations in communicating it to members of the following families of plants: *Cucurbitaceae*, *Scrophulariaceae*, *Papilionaceae*, *Convolvulaceae*, *Caryophyllaceae*, *Cruciferae*, and *Compositae*. In their experiments, infection was secured on various cultivated varieties of tobacco, on several other species of *Nicotiana*, and on a number of other solanaceous genera.

Symptoms.—Ordinarily the disease involves the lowermost leaves of plants in the field. Growers maintain that in seasons when the disease is severe it attacks the entire foliage. It is entirely probable that such is the case, since the top leaves, when inoculated, become infected as readily as do the lower leaves.

The first evidence of infection is the presence of circular, pin-point-like specks surrounded by distinct, chlorotic halos, identical in appearance with those of wildfire. At times there is no halo, or it does not persist, but instead disappears rapidly. The lesions rapidly enlarge, forming spots about a centimeter in diameter, and the diseased tissues become distinctly brown or brownish white. If the infected area involves a vein, the lesion is elongated; otherwise it is circular in outline unless several lesions fuse to make an irregular spot.

The disease has been observed on plants in the seed bed. Here it forms inconspicuous, angular spots which are surrounded by less distinct, chlorotic areas than on leaves of plants in the field. With age the affected tis-

sues become pale brown. Abundant lesions result in a blighting of the foliage of seedlings.

The Disease.—This disease, which is known by growers in Wisconsin as "rust" or "old fashioned rust," was described by Johnson (1923) as "Wisconsin bacterial leaf spot." This name serves to distinguish it from such well-known bacterial leaf spot diseases as angular leaf spot and wildfire. It apparently has existed in Wisconsin for years, where it is of little importance in certain seasons and very prevalent in others. Johnson first observed the disease in 1917 in the vicinity of Madison, Wisconsin, and, in 1919, he identified it from Kentucky. Collections made in Maryland, Ohio, Pennsylvania, and the Connecticut Valley, which appeared to be this disease, proved on further study to be the wildfire disease. Subsequently Wisconsin bacterial spot appeared in Rumania in the Danube Valley, where losses of 40 per cent or more of the crop were reported (Săvulescu and Rădulescu, 1929). It oc-

Fig. 70. Young lesions of the Wisconsin bacterial leaf spot on a cigar-wrapper type of tobacco. (From James Johnson.)

Fig. 71. Fully formed lesions of the Wisconsin bacterial leaf spot on a mature leaf of cigar-wrapper tobacco. (From James Johnson.)

curs also in Bulgaria and in sections of Russia (Tverskoi, 1931).

Cause.—The Wisconsin bacterial leaf spot is due to a yellow organism, which Johnson (1923) described as *Bacterium melleum*. It has been isolated from seedlings in the seed bed and from mature plants. The causal organism is not a vigorous pathogen, since special conditions are necessary for infection. *Bacterium melleum* is a rod-shaped organism, .5 - .8 x 1. - 2.4 μ, that possesses one to several polar flagella. It does not form spores, is Gram negative, and aerobic. Agar colonies are circular, glistening, smooth, convex, viscid, and yellow with opaque centers. It liquefies gelatine, coagulates milk promptly, does not reduce nitrates, is able to hydrolyze starch feebly, and forms neither acid nor gas from carbohydrates. On potato cylinders, a brownish yellow growth is produced.

The organism from pure culture has been inoculated onto leaves under a variety of environmental conditions by atomizing upon tobacco leaves and by needle puncture. These have resulted in typical infections in wounded leaves, being first evident three to five days after inoculation. Practically no infection occurs when the inoculum is applied by spraying the normal leaves with a watery suspension of the organism. While it appears that infection in the field is dependent on favorable environmental conditions, every attempt to duplicate these conditions experimentally in the greenhouse in controlled temperature and humidity chambers has given negative results.

Field observations indicate that the presence of this disease is correlated with availability of the chemical elements in the soil, and that faulty nutrition predisposes to attack. The results of Săvulescu and Rădulescu's (1929) investigation indicate that the strains of *Bacterium melleum* with which they worked were much more virulent than those isolated by Johnson (1923). Injuries to the leaves were not necessary for infection. They secured infections by spraying the leaves with a suspension of the pathogen.

Control.—The only observations available which serve as a basis for recommendations for control are the occurrence of the disease on seedlings in the seed bed. This suggests the employment of sanitary measures which have for their purpose the prevention of the disease. These should include seed disinfection, and the sterilization of seed beds, including frames, covers, and soil.

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Hollow-Stalk and Black Leg

Hollow-stalk is the name applied to one of the minor diseases of tobacco in the field. It has been observed throughout all tobacco-growing districts of the United States and has also been reported from the Dutch East Indies, Japan, and South Africa. The name "black-leg" is applied to a seedling disease of tobacco caused by the same organism (Johnson and Valleau, 1931). No special study of the disease has been made anywhere, and, in consequence, it remains little known. It probably has been recognized by growers for a long time, because of the fact that the pith is involved in a characteristic foul-smelling decay.

The organism which causes hollow-stalk and black-leg of tobacco is identical with one which produces a rot of calla lily *(Zantedeschia aethiopica)*, and a soft rot of green tomatoes. In addition, it has been shown to be capable of producing a soft rot disease of cabbage, carrot, cauliflower, celery, cucumber, eggplant, kohl-rabi, lettuce, muskmelon, parsnip, pepper, Irish potato, radish, salsify, and turnip (Massey, 1924).

Symptoms.—The hollow-stalk germ does not usually attack tobacco until it has reached the topping and suckering stage. The disorder can be recognized by a wilting

of the leaves, usually of isolated plants of a few standing near together in the row. The disease may appear first on the uppermost leaves or upon those near the base of the stalk. In the latter case the leaves collapse and fall to the ground close to the stalk, remaining attached by the petiole or leaf base. Brown, sunken lesions are initiated in the axils of the leaves from which the suckers have been broken out or in the wound made by the removal of the top. Wounds appear always to be necessary to permit the entrance of the pathogen. Progress of the disease is favored by wet weather, when it may be noted that the pith and cortical portions of the petiole rapidly decay. Quite commonly the pith of the entire stem becomes a rotten slimy pulp, which, on drying, leaves the stem quite hollow. Such plants succumb. Black-leg, the seedling form of the disease, may appear during wet periods. The leaves which touch the ground become involved in a wet rot that spreads through the petioles into the stem. The disease may progress until the stems rot off, and in any case the lesion turns black. Seedlings in patches in the bed are destroyed.

The pathogen is at first intercellar and advances by dissolving the middle portion or layer of the cell wall which is of different composition than the portions on either side. It appears to be able to digest the middle layer in advance of actual penetration by the organism. By so doing, the cells are separated from one another. The protoplasm of these cells soon collapses, due no doubt to by-products formed by the pathogen, and in late stages of the disease all parts of the tissues are filled with bacteria.

The Disease.—While it seems likely that this disease is referred to in some accounts of tobacco culture written in colonial days, Johnson (1914) first made brief mention of it using the name "hollow-stalk." He noted it in Wisconsin, but subsequently it has been found in Connecticut, Massachusetts, Virginia, North Carolina, and Kentucky. It is sporadic in occurrence, and has only once in the writer's experience been observed to be of importance.

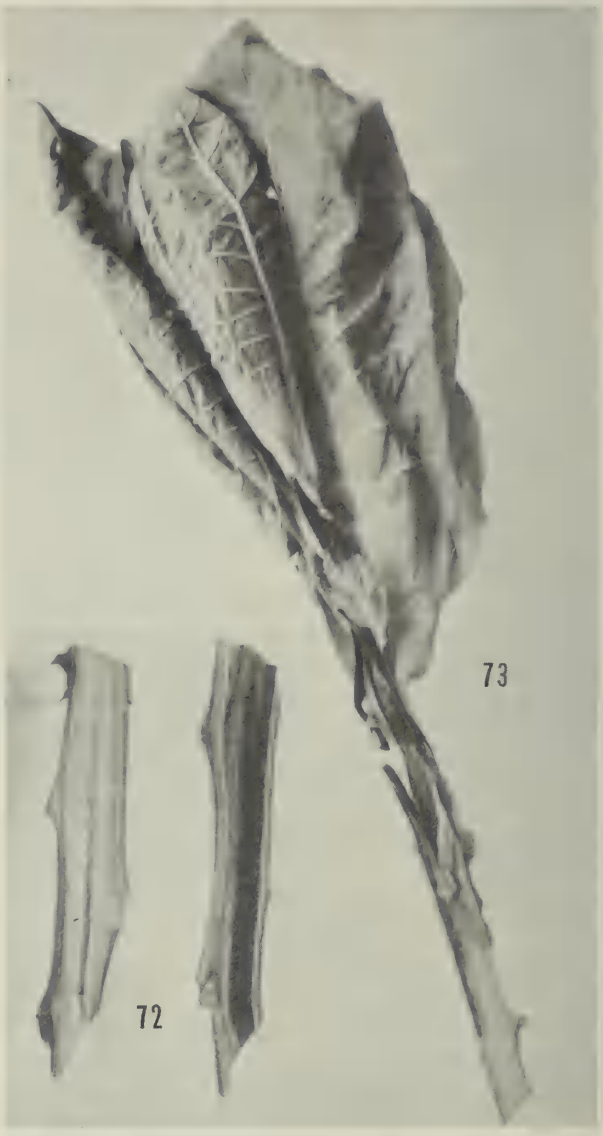

FIG. 72. Portions of the stems of tobacco that have been split. The pith is intact in the healthy one at the left, and has been completely destroyed by the hollow stalk bacteria in the one at the right. (From James Johnson.)

FIG. 73. Split stem of a tobacco plant showing the decay of the pith produced by inoculation with the germ that causes hollow stalk. (From James Johnson.)

In 1928, in the Burley-growing section of North Carolina, where excessive rainfall occurred during the latter part of the growing season, the disease was present in destructive proportions.

Cause.—Hollow-stalk and black-leg are caused by *Bacillus aroideae* Towns., first described by Townsend (U. S. Dept. Agr. Bur. Plant Ind. Bul. No. 60, 1904), as the cause of a soft rot of calla lily. *Bacillus aroideae* is a rod-shaped organism, .7 - 1.0 x 1.5 - 3 μ, with peritrichiate flagella, no spores, and is Gram negative. Agar colonies are white to opalescent, glistening, amoeboid in shape. Gelatin is liquefied, milk curdled, and peptonized with gas formation. Nitrates are reduced; slight diastatic action occurs; and acid without gas is formed from dextose, lactose, sucrose, galactose, glycerol, and mannitol.

Johnson isolated a bacillus of the soft rot type from affected tobacco in Wisconsin with which he was able to

Fig. 74. Black leg on Burley tobacco seedlings. It is caused by the same organism that is responsible for the hollow stalk disease. (From Valleau.)

reproduce the disease and attributed the disease to *Bacillus carotovorus* Jones. Massey (1924) inoculated tobacco stems with *B. carotovorus* with no evidence of infection. When, however, tobacco stems were similarly inoculated with *B. aroideae* isolated from soft-rotted tomatoes, a browning of the pith developed within thirty-six hours. This was followed by a soft rotting and a collapse of the cells. Although both species are capable of causing soft rot diseases and have many points of similarity, yet they are distinct species. Smith (An introduction to bacterial diseases of plants, 1920) regards *Bacillus melonis* Giddings, which causes a soft rot of cantaloupes, as identical with *B. aroideae*. Nakata (1927) made a morphological and physiological study of the organism which causes hollow-stalk, in Japan, and found it to be identical with *B. aroideae*.

Control.—The hollow-stalk bacteria are spread from an occasional diseased plant to neighboring ones on the hands of workers during topping and suckering. If the plants occur on poorly drained soils, or if they are succulent due to continued wet weather, and the plants are handled while they are wet and the weather is cloudy, ideal conditions obtain for spread of the pathogen and the development of the disease. The only precaution of value in such cases is to avoid handling diseased plants until all healthy ones have been topped and suckered. If diseased plants are handled, it is necessary that the hands be cleansed before continuing work on healthy ones. When plants are handled during dry weather rather than during rainy periods, the wounds heal more quickly, and there is much less likelihood that infection will result.

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BLACK RUST

A leaf-spot disease called rust was first briefly described by van Breda de Haan (1898) on Deli tobacco in Sumatra. He observed that affected tissues were filled with bacteria which appeared under certain conditions to be the cause of the disease. Further mention of rust was made by Hunger (1901), but he did not contribute any additional facts to an understanding of the disease. In 1914 Honing made a study of the cultural characteristics of the pathogen and gave it the name *Bacterium pseudozoogloeae*. Meanwhile planters had come to call the disease *zwarte roest* (black rust) in order to distinguish it from another rust which was apparently due to a deficiency of phosphoric acid. Both names are of course inappropriate and confusing, since the term rust should be retained for diseases caused by the true rust fungi. It is perhaps best, however, to retain the name "black rust" for the time being for the disease under consideration.

SYMPTOMS.—The disease is first evident by the presence of dark green leaf spots whose central portion soon become dark brown and zonate with concentric rings. A border of dark green continues to be formed in advance of the central necrotic tissues. This dark green color persists even in the dried leaves. Mature spots vary in size from 1 to 2 cm. Apparently concentric rings are found in the case of no other tobacco disease in the Dutch East Indies, and Jochems (1926) uses this characteristic as a certain means of diagnosis.

CAUSE.—A bacterial organism, to which the name *Bacterium pseudozoogloeae* was given by Honing in 1914, is the cause of black rust. He isolated the pathogen in pure culture and found that it is a non-spore-forming, rod-shaped organism .9 - 2.5 x 0.7 - 1.0 μ, motile by means of 1 to 2 polar flagella. It forms round or oval clumps in

broth, and yellowish gray colonies on gelatine or agar. Milk is coagulated but not cleared by it. Gelatine is liquefied, and nitrates not reduced. No gas is formed, but acid is produced from dextrose, lactose, maltose, sucrose, and mannit.

Inoculations were made by swabbing the upper leaf-surface with cotton plugs soaked in broth cultures. Nine of the eleven plants inoculated by him with one of the isolants became diseased while near-by plants, which were not inoculated, remained healthy. His inoculation experiments and observations when taken together show,

FIG. 75. Black rust lesions on Sumatran tobacco. (After van Breda de Haan.)

however, that the bacterium is incapable of attacking tobacco except under conditions of excessive rainfall and high humidity. Such conditions obtain more commonly in fields several hundred feet above sea level than in the lower-lying plantations. Like other bacterial leaf spots, the organism is spread by splashing rains. Apparently it persists in fields year after year but produces infection and becomes abundant only during exceptionally rainy periods.

Control.—No control measures, based upon experimentation, have been devised. Honing (1914) recommends that the lower diseased leaves be gathered and burned in order to prevent the spread from them to other leaves higher up on the stem. Since black rust becomes epiphytotic only during protracted, very wet seasons, this procedure might have some merit.

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Ryaboukha

Under the Russian name *Ryaboukha* there has been included a number of distinct leaf-spot diseases (Krashnina, 1930, and Miller, 1929), although they were not distinguished as separate entities at first. This name, in the beginning, included such disorders as wildfire, a leaf spot caused by *Ascochyta nicotianae,* and another by a species of *Phyllosticta.* It should, however, be restricted to a bacterial disorder whose causal organism was described by Vzoroff (1930) as *Phytomonas heterocea,* and which resembles most *Bacterium melleum* Johnson.

The disease appeared in the North Caucasus about 1927 on introduced varieties of *Nicotiana tabacum.* In its macroscopic symptoms it resembles very much the Wisconsin bacterial leaf spot. Observations by Vzoroff

(1930) indicate that the pathogen gains entrance through the stomata and is spread by splashing rains.

The causal organism is a Gram negative, non-spore-forming, motile rod, measuring 1 - 2 x .4 - .6 μ. On agar it forms circular, convex, smooth-margined, semi-transparent, glistening, light yellow (later amber-colored) colonies. Their surface is pitted, and there is a large granule in the center. It slowly liquefies gelatine, does not coagulate milk nor form indol, but produces ammonia and sulphuretted hydrogen. It reduces nitrates and hydrolyzes starch. Acids are formed from glucose, sucrose, lactose, maltose, galactose, arabinose, xylose, salicin, glycerine, and mannite. Litmus milk first became acid and later turned alkaline.

Vzoroff by inoculation to uninjured tobacco leaves proved its pathogenicity.

Apparently the ryaboukha disease, which was studied by Kokhanovskaja (1929, 1930), and which he believed to be the most destructive malady of *Nicotiana rustica* in Ukrania and the North Caucasus area, is identical with wildfire. The writer is of this opinion because of Kokhanovskaja's cultural studies and of the fact that it was pathogenic to a long list of species and varieties of Nicotiana.

Control.—Vzoroff (1930) observed that the disease involves the pods, and was able to establish the fact that the seed from affected pods are contaminated. For this reason he recommends disinfection of the seed, and used for this purpose chloride of lime. The organism appears also to survive in débris in the fields, which suggests that rotation of crops is a necessary protective measure.

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PHILIPPINE BACTERIAL LEAF DISEASE

In 1925 Clara briefly described a bacterial leaf-spot disease of tobacco that is not known to occur except in the Philippine Islands. It was first noted in seed beds and later in the field at the Ilagan Tobacco Experiment Station of Isabela. Presumably it had been previously confused with other leaf-spot diseases, such as frog-eye, wildfire, and angular leaf spot, which also occur there.

SYMPTOMS.—The disease, on seedlings, is characterized by the presence of bleached white spots that vary in size from that of a pinhead to that of a "centavo." When the spots are marginal in origin, a wet rot may develop. The damage is most evident and most serious when the infection starts on the petioles or stem. This condition simulates damping off, caused by *Pythium de Baryanum*, and results in the death of the seedlings.

On plants in the field, leaf spots are produced that are white or opaque but sometimes brown and zonate. Young lesions are bordered by a yellow halo similar to wildfire, but this also disappears as the leaves mature. The spots are variable in size, and may become so abundant on a single leaf as to fuse into large irregular areas. Lesions occur most abundantly on the lower leaves. After the crop has been cured, the brown lesions are not easily seen, but the white ones remain conspicuous. In any case, such leaves, if used in the manufacture of cigars, must serve as binders and fillers since they are not suitable for cigar wrappers.

CAUSE.—Clara described the cause of Philippine bacterial leaf disease as *Phytomonas polycolor*. It is a nonspore-forming, rod-shaped organism, 2.67 μ by 1.27 μ, that is motile by means of 1 to 3 flagella. When grown on potato-dextrose agar, the colonies are Primuline yellow

and fluorescent; on potato cylinders, yellowish to sepia brown. In broth, heavy clouding occurs, with the production of delicate flaky membranes. Gelatine is liquefied; nitrate is reduced; starch is not digested; litmus milk is rendered alkaline; acid is formed in glucose, xylose, mannose, and arabinose, but not in lactose or saccharose broths; no gas is formed in carbohydrate media.

The pathogenicity of *Phytomonas polycolor* was established by several series of inoculations in which the organism was applied through punctures, by smears on the leaf surface, or by atomizing. Infections were secured with both methods of inoculation, but the latter was the least successful. Under most favorable conditions infection is first evident after two days. Mature or overmature leaves, the lower ones, readily became infected, and the youngest leaves were rarely infected.

Control.—Observations indicated that the disease is carried to the field on infected seedlings. Lesions were also noted on the capsules of severely infected plants. These two observations suggested that the disease is seed borne. This conclusion was established by germinating seed from affected plants and noting that the disease appeared on the seedlings. Because of this evidence, furthermore, Clara used silver nitrate, 1:1000, and mercuric chloride, 1:1000, in seed disinfection tests during two seasons, and appreciably reduced the disease. The former disinfectant appears the better under Philippine conditions, and seed treatment is recommended by him as a primary control measure. This procedure should be supplemented by other sanitary measures, since the pathogen is known to remain alive in old leaves for a period of at least a year.

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Crown Gall

The name "crown gall" is usually applied to overgrowths that appear on plants in the region where the

root and stem join. Tobacco is not known to be affected with crown gall under field conditions, but it has been observed by numerous workers to be subject to attack under experimental conditions. Voluminous publications have appeared that show a similarity of crown gall and animal cancers, especially human cancer. Our knowledge of crown gall is largely the result of the investigations of Smith and his associates (1911, 1912, 1922).

Suscepts.—Crown gall is known to occur on a wide variety of plants, both woody and herbaceous. Smith and others (1912) isolated its causal agency from 24 species belonging in 14 different families. The species include such woody plants as apple, peach, pear, willow, grape, rose, blackberries, and raspberries, and such herbaceous ones as alfalfa, clover, cotton, tobacco, sugar beets, hops, and daisy.

Kostoff (1930) inoculated hybrids of several species of *Nicotiana* and found that not all manifested tumors and other overgrowths. When, for example, the progeny of *N. glauca* X *N. langsdorffi* or of *N. tabacum wigandioides* X *N. sanderae* were inoculated, the overgrowths were frequently so abundant that many of the plants died before reaching maturity; while that of *N. tabacum* X *N. glauca, N. glauca* X *N. sanderae, N. sanderae* X *N. langsdorffii, N. tabacum* X *N. paniculata, N. rustica* X *N. paniculata,* or *N. paniculata* X *N. glauca* never manifested any abnormal proliferations.

Symptoms.—As the name indicates, this disease is manifest by the formation of gall-like enlargements that are for the most part composed of parenchyma tissue. Instead of killing the cells as is the case with all other bacterial diseases of plants, the cells are induced to multiply, a hyperplastic reaction, until large masses of cells are formed. Sometimes these overgrowths are many times larger than the stem on which they are borne.

Another form of response in tobacco to the crown gall organism is the formation of fasciations and witches' brooms. The fasciations arise as quite normal structures but soon change into juicy, flat, concrescences that bear

narrow succulent leaves. They usually form in the region where the old leaf has fallen off. In witches' brooms, the bud tends to remain embryonic, and multiple buds arise on the primary bud before it has had opportunity to become differentiated into a leafy branch. The whole branch in consequence is atrophied. All possible gradations may occur between slight fasciation and well-developed witches' brooms.

Cause.—The primary cause of crown gall is *Bacterium tumefaciens* Smith and Townsend. It is a rod-shaped organism, occurring in chains, 0.4 - 0.8 x 1 - 3 μ. It is aerobic, non-sporiferous, and Gram negative. Colonies on agar are small, circular, raised, glistening, white, and translucent. It does not liquefy gelatine, digest starch, reduce nitrates, nor form gas from carbohydrates. Acid is formed from dextrose and sucrose; curd is formed in milk, but this curd is not digested by it. *Bacterium tumefaciens* accomplishes the production of malformations not alone by means of substances which it produces, but also by means of "necrotic products of the cells in the af-

Fig. 76. Crown gall on tobacco. Inoculations with *Bacterium tumefaciens* were effected in or near the axils of the leaves. Globular galls bearing numerous leafy shoots developed. (After Levine.)

fected regions as well as catabolic products resulting from abnormal metabolic processes in the cells under the influence of the bacterial substances'' (Kostoff, 1930). Smith has shown that such chemicals as formic acid, acetic acid, ammonia, and aldhyd are all capable of stimulating hyperplasias in plants that resemble tumors. These findings have thrown a flood of light on the proximate cause of malignant growths.

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LESS WELL-KNOWN BACTERIAL DISEASES OF TOBACCO

PELLAGRA.—A diseased condition of the root system of tobacco in Sicily and parts of Italy has been described by Comes, whose contributions to the history and classification of *Nicotiana* are classic, as due to *Bacillus gummis*. This disease does not appear ever to have been noted elsewhere. It manifests itself by a blackening and decay of the root system and the formation of gum in the discolored xylem portions. New roots tend to form just beneath the surface of the soil. The entire plant remains stunted and appears undernourished, bearing pale green, narrow leaves.

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CHANCRE.—In a preliminary report Delacroix (1903) called attention to a diseased condition of young tobacco plants, 8 to 12 inches tall, which manifested itself by the presence of oblong, discolored, sunken areas on the stems

and principal veins. The central portion of these lesions became dry and white with age. When several diseased areas appeared on the stems in close proximity, the stem lost its rigidity, and the leaves wilted. He ascribed these cankers to an organism which he named *Bacillus aeruginosus* Delacroix. Whether it is pathogenic is not known, but it is probable that it is not the cause of these cankers and that it is synonymous with *Bacillus fluorescens liquefaciens* or with *B. fluorescens putridus.*

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WHITE SPECK.—This name is used to designate a diseased condition of well-developed or mature leaves of tobacco, which manifests itself as white spots. Although it was first mentioned in 1894, no one has made a thoroughgoing investigation of white speck. Its known range includes France, Italy, Switzerland, Greece, and Bulgaria.

The diseased areas are at first yellowish green and become circular to irregular white spots 3 to 8 mm. in diameter. The margin is brown, raised, and corky. Sometimes the spots fuse to form irregular areas which, with age, may fall out. Savoff (1924), who noted that this disease caused severe injury in Bulgaria in 1923 and 1924, stated that the lesions resemble those produced by *Phyllosticta tabaci* Pass. except that this fungus forms dark pycnidia within the gray spots.

The pathogen responsible for white speck is *Aplanobacter maculicola* (Delacroix) Elliott. It is a non-flagellate rod, 1.5 x .75 μ, which does not form spores and is Gram negative. Colonies on agar are small, iridescent, glistening, cream-colored at first, and later become bluish. It is capable of liquefying gelatine with the production of an odor like nitrobenzine.

Savoff (1924) isolated it and proved its pathogenicity. Cavadas (1927) noted that a moth, *Gnorimoschema heliopa,* was an agent of its dissemination in Thessaly.

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COLLAR ROT.—This name applies to a disease that manifests itself by a blackening of the lower portion of the stem, at or near the ground level. The discoloration gradually extends upward, the leaves wilt, and ultimately the plant dies. The disease, as first described by Delacroix (1906), was ascribed to *Bacillus tabacivorus* Delacroix. It is, however, a non-motile rod and therefore does not properly belong in the genus *Bacillus*. The rods measure .75 - 1.5 x .4. - .6 μ. The organism is aerobic, does not form spores, and does not liquefy gelatine. Colonies on agar are dull, slightly elevated, and pale yellowish-white; on potato cylinders they are white; on gelatine they are white also but with a brilliant, metallic, iridescent lustre. Its pathogenicity has not been demonstrated, and its range includes France and Bulgaria.

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VIII

Diseases of the Growing Crop Caused by Fungi

Characteristics of Fungi

Most diseases of plants are caused primarily by microscopic plants called fungi. The most characteristic feature of fungi is their inability to make their own food. All green plants are able to elaborate their own food by virtue of the possession of green pigment called chlorophyll. Fungi do not possess chlorophyll and must, therefore, depend for their supply of food upon other plants or upon animals. Some fungi obtain their food from living plants or animals, and this group, in consequence, is termed parasites. Others are entirely unable to take food from living tissues but can feed only upon dead or decaying parts of plants or animals, and this group is termed saprophytes.

Certain fungi can be grown only when in contact with living cells, as for example, the fungus responsible for the powdery mildew of tobacco, the one that causes tobacco rust, and the one that causes downy mildew or blue mold of tobacco. Such fungi are commonly spoken of as obligate parasites. Others, as the tobacco black shank fungus, the Fusarium wilt fungus, the sore shin fungus, and the black root rot fungus, attack the growing crop, and after the death of the plants, can accomplish their decay and subsist upon these products of decay for a period of a year or more. These fungi are facultative parasites or facultative saprophytes.

The vegetative portion or stage of fungi is usually thread-like. Each thread-like element is a hypha, and the entire aggregation of branching hyphae is known as the mycelium. The fruiting or reproductive stage of fungi

consists of spores, which on germination are capable of giving rise again to the vegetative stage. Each fungus produces typically one or more types of spores. The type of structure in which or upon which the spores are formed, the shape of the spores, their color, size, septation, surface markings, etc., are characteristics that enable one properly to classify fungi.

Parasitic fungi are capable of producing diseases in plants, and most of them possess a spore stage that appears upon the diseased parts and serves for the rapid dissemination of the fungus to other susceptible plants. Toward the close of the growing season or after the death of the affected parts, another spore stage may form that serves to enable the fungus to hibernate. The tobacco powdery mildew fungus and the one that causes pleosporose are known to possess a spore stage that survives the winter period. Others hibernate or remain dormant for a time by means of sclerotia, which are structures that consist of compact masses of vegetative parts, as is the case with the sclerotium blight fungus, the *Sclerotinia*-disease fungus, and the sore shin fungus.

Obligate parasites like the fungus that causes downy mildew of tobacco cannot be grown in culture on synthetic food substances. This is one of the reasons that it is difficult to investigate diseases that are caused by obligate parasites. Practically all facultative parasites, however, may be cultured in the laboratory with more or less facility. Some of them may be induced to develop all of their vegetative and reproductive stages in culture, as is the case with the tobacco black shank fungus, the gray mold fungus, the *Olpidium* seeding-blight organism, and the fungus that causes black root rot. Others, as the sore shin fungus, the one that causes sclerotium blight, and the Sclerotinia-disease fungus, bear no spore stages in culture but form an abundance of mycelium and sclerotia.

Some of the fungi that attack tobacco occur on seedlings, as is the case with *Olpidium brassicae* and species of *Pythium*. Others, however, can attack plants in any stage of their growth and may even injure the crop during

curing, as is known to be the case with the frog-eye fungus and the black shank fungus.

The occurrence of specific fungous diseases of tobacco in destructive proportions is probably closely correlated with favorable weather conditions. Exact data in support of this statement are, in most cases, lacking, but observations indicate that this is generally true. The best known cases include the gray mold disease and the downy mildew of tobacco seedlings whose occurrence is correlated with subnormally cold springs with excessive rainfall. Temperatures of 45° to 60° Fahrenheit at times when the sky is continuously overcast for several days, with intermittent rains, is ideal for the development of the downy mildew or blue mold disease. The fact that such diseases as frog eye may be very prevalent one season and of little or no importance in the succeeding year is most likely related to weather conditions. Frequent rains favor its presence. All too little is known of the relationship of environmental conditions to diseases of crop plants, and the bearing that weather may have upon the occurrence of a particular disease in epiphytotic proportions in one section or country and upon its presence as a minor disease in another.

Black Root Rot of Tobacco

The black root rot disease of tobacco is among the best known of the diseases of this crop. Numerous investigations of it have been conducted within the United States in Wisconsin, Connecticut, Pennsylvania, and Kentucky, and within Europe in Italy. Its cause is a soil-inhabiting fungus whose growth is strongly modified by soil conditions, notably soil reaction and soil temperature. The causal fungus is not confined to tobacco, but can attack a wide variety of both wild and cultivated species of plants comprising about a hundred species in widely separated families.

Suscepts.—The first report of black rot on tobacco was made by Peglion (1897) in Italy. Apparently Killebrew had previously noted the disease in Pennsylvania

since his description of a disease involving the roots of tobacco, in 1884, accords with that of the black root rot disease. It is certain that Selby (1904) encountered it in Ohio in 1903.

The causal fungus was first collected on pea *(Pisum sativum)* in England in 1850. Johnson (1916 b) regards it as pathogenic primarily to legumes, as shown by his own investigations and those of numerous others. Among the leguminous species which, from the early investigations, it is known to attack are *Lupinus angustifolius, L. albus, L. thermis, L. luteus, Trigonella coerulea, Onobrychis crista-galli, Trifolium repens* (white clover), *T. pratense* (red clover), *T. hybridum* (alsike clover) *Medicago sativa* (alfalfa), *Vigna sinensis* (cowpea), and *Phaseolus vulgaris* (bean). It occurs also on such cultivated plants as watermelon, violets, and cotton. Johnson's studies (1916 b) on susceptibility revealed that 66 additional species including 28 *Leguminosae*, 20 *Solanaceae*, 7 *Cucurbitaceae*, and 11 species belonging to various families are to be regarded as susceptible to attack by the tobacco black root rot fungus. In the *Leguminoseae* are included, in his findings, such important crops as peanuts *(Arachis hypogoea)*, soybean *(Glycine hispida)*, lespedeza *(Lespedeza striata)*, bur clover *(Medicago denticulata)*, sweet clover *(Melilotus alba)*, and crimson clover *(Trifolium incarnatum)*; in the *Solanaceae*, *Nicotiana glauca, N. sylvestris, N. sanderae, N. repanda, N. atropurpurea, N. langsdorffii, N. glutinosa, N. chinensis, N. macrophylla, N. alta, N. calyciflora, N. angustifolia, N. latterrima*, and *N. longiflora*.

Varietal Resistance.—Peglion (1897) noted that the "Seed-leaf" type of tobacco in Italy was less injured by black root rot than Kentucky Burley. Beninceasa (1902) also observed that the varieties which had been developed in Italy were more resistant than Burley. A variety known as *Brasile Beneventano* was found to be the most resistant. In 1911 he (Beninceasa, 1911) called attention to the resistant qualities of a hybrid between *Salento* and *Italia*. Aielli-Donnarumma (1911) reported the re-

sults of his experiments on hybridization to develop varieties resistant to root rot, finding that "Italia x Kentucky B" was strikingly resistant.

Johnson (1916 a) tested the susceptibility of these resistant Italian varieties along with 21 other varieties in Wisconsin in 1915, and found a range of relative resistance from 2.9 to 78.7 per cent. Such standard varieties as White Burley, Orinoco, Pryor, and Maryland varieties were found to be most susceptible. Little Dutch and Connecticut Broadleaf tobacco proved to be most resistant. By selection he was able to develop a strain of White Burley which possesses a high degree of resistance. Subsequently selections of White Burley from Kelley's Stand-up and Judy's Pride were developed in Kentucky, and are widely grown in the Burley-tobacco sections of Kentucky, Tennessee, and North Carolina. These varieties are now being improved, and the highly resistant hybrids used in their stead. In the Connecticut Valley, among cigar-tobacco types, strains of Connecticut Broadleaf, Cuban, and Little Dutch have been developed that are very satisfactory.

With the appearance of black root rot in serious proportions in North Carolina on flue-cured tobacco, experimental work on control was begun at the Tobacco Experiment Station, Oxford, N. C. This work resulted in the production by selection of a strain of Paris Wrapper and one of Jamaica that are highly resistant and that are very satisfactory in yield and quality.

The Disease

Names, History, Range, and Importance.—Various names have been applied to this disease, including *marciume radicale*, "root rot," "Thielavia root rot," and "black root rot." Since there are several diseases that involve the roots of tobacco, and since the affected roots are characteristically blackened, the name "black root rot" is to be preferred.

Black root rot has undoubtedly been known by planters in Virginia and in the Connecticut Valley as long as

tobacco has been cultivated there. The first authentic description of the disease appears to be that of Killebrew (1884). Its cause was not established, however, until 1897 by Peglion, who noted its prevalence in seed beds in North Central Italy. The disease is now known to occur in all of the tobacco-growing regions of the United States, Canada, and Europe. It has also been reported to attack tobacco in Japan and Australia.

The effects of black root rot in any particular field vary from little or no appreciable damage to the death of many plants. The plants may succumb shortly after they have been transplanted or while they are still in the seed bed. Such plants as survive may be severely stunted. Some idea of its importance may be gained from the opinions of those who have made field studies of the disease. Johnson (1916 a) states: "The losses conservatively can be placed at between ten and twenty millions of dollars for this year in the United States alone." Johnson and Hartman (1919 a) regard it as "the most serious disease with which tobacco growers in most producing sections have to contend." Anderson and associates (1926) believe that "black root rot probably causes greater losses to the tobacco growers of the United States than any other disease."

Symptoms.—Black root rot is primarily a root disease and can appear on tobacco at any stage of its growth. In general, it is characterized by a blackening and decay of the tap root and lateral roots of seedlings and also of the larger plants in the field.

A. *In the Seed Bed.*—If attacked when very young, the seedlings are subject to a typical damping off. The lesions are formed not only at the soil level but may extend up to the cotyledons and downward into the lateral roots. If plants are somewhat older when attacked, or if infection is not so severe, the roots alone are infected and the plants are stunted. The tips of the rootlets are blackened and dead, and in time this necrosis progresses up the laterals into the tap root and thence into the branch roots formed higher up on the stem. Seedlings pulled from the

soil at this stage show only the blackened base of the stem remaining. This symptom-complex may be accompanied in later phases by the proliferation of adventitious roots above the lesions. These new roots may serve to supply the plant for a time, but they eventually become involved as the lesion is extended. Meanwhile, symptoms appear on the above-ground portions of the seedlings. In soils well supplied with nitrogen the only visible symptom is a slightly deeper green of the leaves. In depleted soils, however, the stems and leaves are stunted. Such plants are pale green, the yellowing begining at the leaf tips and

FIG. 77. An entire plant that has been stunted by black root rot in comparison with the basal part of a healthy plant of the same age. Note the differences also in the extent of the root systems. The roots are blackened and rotted off by black root rot. (From James Johnson.)

progressing toward the petiole. In affected beds the growth is very uneven, large plants occurring in some portions, and in others the plants are small and scattered, and their color varies from an intense green to a completely yellowed condition.

B. *In the Field.*—If diseased plants are set in the field, or healthy plants are set in a field whose soil is infested, they usually show no apparent growth for two or three weeks. Meanwhile many of them will have become yellow, wilted, and have died. In light, open-textured soils the plants that survive gain ascendancy over the disease and eventually produce a fairly marketable late crop. In heavy soils, however, the plants may become permanently dwarfed and produce few if any marketable plants. Sometimes whole fields have to be reset about two weeks after transplanting because the plants have either perished or have failed to make any growth. If the season is cool, a third resetting may be necessary before a stand can be secured.

Cause of Resistance.—Conant (1927) made a careful study of seven varieties of tobacco showing a gradient from high susceptibility to high resistance to black root rot. The plants of each variety were grown in sterilized soil, and in soil inoculated with the root rot organism, and were kept at 20°, 25°, or 30° C. continuously for six weeks. He found that healthy roots of susceptible varieties show a "lag in the initiation of pericyclic division behind cambial development, resulting in an unprotected primary cortex, and especially in an unprotected gap at the point where branch roots have ruptured through the cortex." It required the higher temperatures to stimulate cork formation in susceptible varieties, while it occurred at all temperatures in resistant ones.

Conant (1927) was also able to show a definite correlation between resistance and cork formation in the tissues underlying the lesions. The primary cortex of all but the most resistant variety allowed easy fungal penetration. The entrance of the fungus into the cortex stimulates premature pericyclic division in the young roots

of the resistant varieties. Furthermore, under the stimulation of the black root rot fungus, all secondary root tissues of resistant varieties, except the dead xylem elements, were able to "cork out" the invading mycelium.

Cause.—The organism responsible for the black root rot disease of tobacco is commonly regarded as *Thielavia basicola* Zopf. It is supposed to possess three stages, an ascoporic, that rarely occurs in nature and that has been reported in culture only by Peglion (1897) and McCormick (1925), an endoconidial, and a chlamydospore, both of which stages are always present on lesions. These two imperfect stages are classified as *Thielaviopsis basicola* (Berk.) Ferraris. The relationship of ascosporic, endoconidial, and chlamydospore stages has never been established. A brief historical résumé of the pathogen has been prepared by McCormick (1926) in which she states:

In 1850, Berkeley and Broome found the chlamydospores at the base of stems of peas and of *Nemophila auriculata* and gave the fungus the name of *Torula basicola* n. sp. In 1876 Zopf published an article in which he described a fungus growing on *Senecio elegans* L. in the Botanical Garden of Berlin and he recognized the chlamydospores as identical with those described and figured by Berkeley and Broome. In addition to the chlamydospores, he described endoconidia, ascospores, and spermatia. The last spore form has not been observed by other workers and Zopf himself omitted it from a later publication. Due to the association of perithecia with the chlamydospores and endoconidia Zopf, in his publication in 1876, changed the name, *Torula basicola*, given by Berkeley and Broome, to *Thielavia basicola* and placed the fungus in the Perisporiaceae. Since the date of that publication the fungus bearing chlamydospores and endoconidia, noted above, has also been known as *Thielavia basicola* Zopf and the perithecia occasionally found with them have been considered the perfect stage of the fungus. In August, 1876, Sorokin announced the finding of a new fungus on the roots of *Cochleria armoracia*. He described the chlamydospores but, not recognizing them as belonging to the fungus found by Berkeley and Broome twenty-six years before, nor knowing of Zopf's discovery, named the fungus *Helminthosporium gracile* sp. n. Saccardo lists the fungus in three different places and under as many different names. He retains the name for the

chlamydospore stage given by Berkeley and Broome, but changed the name *Helminthosporium gracile* Sorok. to *Clasterosporium gracile* (Sorok.) Sacc. He also gives a description of *Thielavia basicola* Zopf including in it perithecia with the other spore forms.

Her studies (McCormick, 1925) show that *Thielavia basicola* when grown alone in culture has a tendency to produce perithecia, but is greatly stimulated by being grown with *Thielaviopsis basicola*. This stimulatory effect was also manifest when *Thielavia basicola* was grown with certain other fungi including *Aspergillus umbrinus* Patters., *Cladosporium fulvum* Cke. et Rav., and *Saccharomyces cerevisiae* Meyen. This indicates, as she states, that *Thielavia basicola* and *Thielaviopsis basicola*, although commonly associated, are distinct fungi, the latter being the cause of black root rot.

Pathogenicity.—Various workers have been led to conclude that the black root rot fungus is pathogenic. Peglion (1876) recognized the parasitic and saprophytic modes of life of the fungus, but stated that the extensive injuries to tobacco seedlings might probably be attributed in part to improper cultural conditions. Benincasa (1902) and Selby (1904) both considered the fungus actively pathogenic to tobacco. Gilbert (1909) concluded that virulence of attacks by *Thielavia* varies with environmental conditions, but there can be no doubt of its parasitism on tobacco plants in the seed bed and in the field. He employed seedlings growing in sterilized Petri dishes on blotting paper. Those that were watered with sterilized water remained perfectly healthy until the termination of the experiment, while other seedlings, treated similarly except that the water with which they were moistened contained a suspension of *Thielavia* spores, began to damp off in a few days, and at the end of two weeks every plant had been killed. Examination of the plants attacked showed the presence of *Thielavia* in every instance.

He also autoclaved pots of soil and seeded them. Two days later he inoculated one set of pots by watering with

a suspension of *Thielaria* spores. After a period of four months examination disclosed that the check plants were healthy, whereas the plants from the inoculated pots were diseased with their roots blackened and with *Thielaria* present in abundance.

Conditions Conducive to Black Root Rot.—Gilbert (1909) concluded that the several factors that are conducive to serious injury from this disease are infestation of the soil both of the seed bed and of the field, planting tobacco in soils rich in humus, the application of excessive amounts of commercial fertilizer, excessive watering of the seedlings, and lack of proper ventilation of the beds.

Johnson and Hartman (1919 b) found that soil temperature is undoubtedly the most important factor that determines the extent of the disease. Temperatures of 17° to 23° C. were most favorable for the development of the disease. At temperatures above 26° C. there was little or no infection even in most heavily infested soils. This effect was substantiated from records of soil temperature in tobacco fields. These records showed that the occurrence of black root rot is determined, under practical conditions, primarily by soil temperatures.

Anderson and associates (1926) and Doran (1929) noted that in the Connecticut Valley soil acidity is of prime importance, since in soils with a pH value of 5.6 or lower there was little or no black root rot at any temperature. Marked injury from black root rot began at pH 5.7 at 15° C.; at pH 5.7 to 5.8 at 18° C., at pH 5.8 at 21° C., and at pH 5.8 to 5.9 at 27° C. There was little, if any, injury at 30° C. in soil with an acidity of pH 6.0 to 6.9.

Manifestly both soil temperature and soil reaction are important. These factors, when combined, undoubtedly kept the black root rot fungus from becoming a menace to the growers of flue-cured tobacco in the southeastern United States for a long time. When, however, the soils were limed, as has been practiced for the past ten years or more, and were thus made alkaline or only slightly acid, conditions were made favorable for the development of the black root rot disease.

FIG. 78. A field of flue-cured tobacco grown on a heavily infested soil that had been limed. The stand is broken as a result of the death of over half of the plants. Those that have survived are of very different sizes.

FIG. 79. Field of flue-cured tobacco infested with the black root rot fungus. Resistant strains were selected by saving the seed from the most vigorous plants that came into blossom earliest.

Control.—The foregoing account has indicated that there are two fundamental procedures that are basic to the prevention and control of black root rot of tobacco. The first of these is the sterilization of the seed-bed soil; the second, the use of resistant varities.

Seed-bed sterilization is essential because (1) the causal fungus is one of the soil-inhabiting organisms that can persist for years on decaying matter or can parasitize other plants of many different species, and (2) the transplanting of affected seedlings will result in a seriously diseased crop and in a generalized infestation of the fields. The writer has seen fields whose yields were reduced 25 to 50 per cent as the result of being set with seedlings from infested beds, whereas in previous years these same fields had always borne normal crops.

Highly resistant strains of the several types of tobacco grown in the United States have been obtained by selection and breeding. By similar procedures resistant varieties have been developed in Italy. Use should be made of these strains wherever the black root rot disease is of consequence. This method, as has been pointed out by Johnson (1916), is the most satisfactory and most practical for the control of the disease.

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BLACK SHANK

This disease of tobacco has been the subject of investigation in the East Indies for over forty years. More recently several reports of studies of it in Porto Rico and Florida have added to our knowledge of the disorder.

SUSCEPTS.—van Breda de Haan (1896), who first described the pathogen on tobacco, noted its occurrence also on Amaranthus. Palm (1924) recorded its presence, in addition, on castor bean (*Ricinus communis*), tomato, and two weeds (*Commelina nudiflora* and *Trema ambioensis*). Tisdale and Kelley (1926) successfully inoculated tomato, potato, eggplant, and castor bean with the black shank fungus.

The occurrence of varietal resistance in *Nicotiana tabacum* was first pointed out by d'Angremond (1919). Tests of varietal resistance by Tisdale and Kelley (1926) in Florida, and by Nolla (1928) in Porto Rico, show the existence of differences in resistance and that none is immune. Their trials included many cigar-wrapper and filler tobaccos together with hybrids of these varieties.

FIG. 80. Shade-grown tobacco in Florida affected with black shank. The crop in the rows at the left had been killed, and the disease was spreading along the rows away from the portions shown in the foreground. (From Tisdale.)

FIG. 81. Field of flue-cured tobacco, in North Carolina, in which almost all of the many varieties tested have been killed or have been attacked by the black shank fungus. The row toward the left, with apparently healthy plants is a dark-fired type that exhibits a considerable degree of resistance to black shank.

The selection of resistant individuals among these varieties or crosses enabled them to secure resistant strains.

Tisdale (1931), after selecting and selfing Big Cuba, a wrapper variety, for six years, obtained a strain that possessed a high degree of resistance when grown on thoroughly infested soil. By a like process he secured resistant strains of Little Cuba, Dubek (a variety from Russia), and Santiago (a variety from Java). The yield from the resistant strains of these three varieties, however, is too low to be profitable when grown under shade in Florida. He also selected and selfed strains obtained by crossing Big Cuba and Little Cuba, and secured plants with leaf-characters that represent a blend of both parents.

Experience with a large number of agronomic varieties of flue-cured tobacco in North Carolina shows that but few possess any degree of resistance. Several dark-fired tobacco varieties are highly resistant, however, and *Nicotiana rustica* is practically immune.

Names of Diseases and Importance.—Among the other names by which black shank is known in the Dutch East Indies are "*Phytophthora* disease," *lanas-ziekte*, and *bibitziekte* (seedling disease). In Porto Rico the provincial terms *pataprieta* and *faitapia* are used, evidently in imitation of the English pronunciation of *Phytophthora*.

Black shank was first reported from Java in 1895 and is still one of the major diseases there and also in Sumatra. It was probably introduced into Florida about 1915, according to information gained from growers, although no one knows its source of introduction. By 1925 it had spread to the extent of being prevalent throughout the entire Georgia-Florida cigar-tobacco district. In 1924 it was reported from Porto Rico, Alabama, and Virginia. Subsequently it has been established that black shank does not occur in Virginia. In 1931 it was found to be present in the vicinity of Winston-Salem, N. C., and has not as yet been found in any other portion of the state. It had probably been present there since about 1921, and had spread slowly. How it was introduced into North

Carolina is only a matter of conjecture, and its occurrence in the section long known for the excellence of its flue-cured tobacco has led to the conjecture that it may have been introduced maliciously.

Once black shank has been introduced it spreads with astounding rapidity in warm climates. Total losses occurred in many fields in Florida (Tisdale and Kelley, 1926) in 1924 during the first season that the disease was noted in these fields. As a result, the fields were abandoned to tobacco culture, and in 1925 the acreage devoted to shade-grown tobacco was decreased about 60 per cent.

Recent reports record the occurrence of black shank in Mauritius, Rumania, Bulgaria, Argentina, Uganda, Trinidad, Japan, and Formosa.

Severe losses to seedings occur year after year in the East Indies and in Porto Rico. This form of the disease has been directly responsible for minor losses in Florida and none in North Carolina, due presumably to cool weather throughout the period that the plants are in the seed beds.

Symptoms.—All parts of the tobacco plant in any stage of its development are subject to attack, although the disease involves primarily the base of the stem and the roots. Seedlings "damp off" or become decayed near the soil level and collapse. Such plants, during periods of high humidity and excessive rainfall, soon are entirely involved in a wet rot: during dry weather the tops become dry and brown. If the seedlings are healthy when transplanted, they may become well established in the field before the first evidence of infection is apparent. Plants in spots may then suddenly wilt, and there is no recovery, during the night, from this wilting. Instead, the leaves become yellow, shrivel and dry, beginning with the lowermost. Within a week after symptoms are first apparent the plants will have died. An examination of the underground parts of the wilted plants reveals dark brown to black lesions on the main roots and crown. These lesions may involve all of the roots, or only the smaller ones, or, in some cases, there may be little decay

FIG. 82. Upper part, a portion of a field of tobacco, in North Carolina, grown on newly cleared land. The bare area marks the site of a seed bed in the previous season. Black shank destroyed the plants on the bare area early in the season, and the dying plants around the border show that the causal fungus is being spread through the soil. Lower part, a field in which black shank resulted in the complete destruction of the crop. The black shank fungus was carried from this field to the seed bed shown above, by soil clinging to the shoes of the workmen during the transplanting season.

of the root system. The blackened areas extend up the stalk 6 inches to 1 foot on large plants attacked rather late in their development, or the entire stem of young plants may be blackened.

During rainy periods large, brown, concentrically zonate patches appear on the leaves, especially the lower ones. They are at first pale green, and as the disease progresses, the centers become brown. At such times, especially if the leaves are in contact with the soil, a sparse grayish covering, consisting of the fruiting branches of the causal fungus, appears on the surface of the necrotic parts. When leaves from an infected crop are harvested, large blotches may appear during the first two or three days of curing and render the leaves worthless for wrappers.

When a diseased stem is split lengthwise, it may be noted that the pith is dried for a distance of a few inches to a foot in advance of the cortical lesion, and that disc-like plates of collapsed cells remain in the pith cavity. Dark streaks extend to the cortex beyond the externally visible margin of the lesion. Microscopic examination shows that the mycelium of the pathogen occurs both within such discolored cells and between them (Nolla, 1928). The writer has observed that the mycelium is much constricted in penetrating the walls of xylem tissues. The disease is of the necrotic type. All tissues are subject to invasion, and all are discolored. The vascular tissues do not collapse to the extent that the parenchyma does.

Wolf (1933), in studies to determine the cause of the sudden wilting and collapse of infected plants, demonstrated that it is due to a toxin. This was shown by testing (1) the extract from macerated diseased tissues and (2) the liquid culture medium in which the pathogen had been grown. Both fluids were capable of producing a permanent collapse of healthy seedlings within one to two hours. This toxin proved to be thermostable and non-volatile. He also determined that the pathogen is able to utilize starch, sucrose, and dextrose. T. P. Car-

los, working in the writer's laboratory, found that this fungus can dissolve the middle lamella of invaded tissues and also the cellulose membranes.

Cause.—The fungus that causes tobacco black shank was described by van Breda de Haan (1896) as *Phytophthora nicotianae*. In 1913 Dastur (1913) described, from India, an organism pathogenic to castor bean but not to tobacco, which, because of non-pathogenicity of tobacco, he regarded as distinct from *Phytophthora nicotianae*. Palm (1923), however, inoculated castor bean with *P. nicotianae* and obtained infections. Tisdale and Kelley (1926) were able to confirm Palm's findings on this point, since castor beans were equally susceptible to infection by *P. parasitica, P. nicotianae* from Java, or by the Flor-

Fig. 83. Decay and discoloration of the roots and basal portions of the stems, characteristic of the black shank disease.

Fig. 84. Stems of tobacco plants, affected with black shank, that have been split open to show the drying of the pith in diaphragm-like plates, as may nearly always be noted in affected plants.

ida black shank fungus. The disease was similar following inoculations with each pathogen, and appeared like that described and illustrated by Dastur. Also the cultures from these three sources exhibited only minor differences in growth characters, differences that are clearly not of specific rank. Furthermore, each was capable of producing black shank when inoculated into tobacco, although they were not equally virulent. Leonian (1925) made a cultural study of various strains and species of *Phytophthora,* and noted that *P. nicotianae* closely resembled *P. parasitica.* He proposed that these forms be regarded as *P. omnivora* DeBy.

More recently Ashby (1928) made comparative studies, on rolled oat agar and on maize meal agar, of strains of *P. nicotianae* from diseased tobacco in the East Indies, in Florida, and in Trinidad, and from cotton in Trinidad and *Pollia* sp. in Sumatra. All were like *P. parasitica* as described by Dastur except in size of oospores. He proposes that *P. nicotianae* be merged with *P. parasitica,* with two sections. Those with small oospores, less than 20 μ in diameter, should be placed in the section *microspora.* These include Dastur's strain from castor bean, Sherbakoff's *P. terrestris,* causing buck-eye-rot of tomato, the citrus foot-rot fungus from Florida and elsewhere, the cotton fungus from Trinidad, the tobacco fungus from Porto Rico, the cocoanut bud rot fungus, the organisms causing pineapple and eggplant rots, and *P. allii,* that causes a rot of onions in Formosa. Those with oospores larger than 20 μ in diameter, he places in the *macrospora* section. These include the strains from tobacco from Florida, Sumatra, Java, Trinidad, the strain from *Pollia* sp. from Sumatra, those from cotton from Trinidad, and *P. parasitica* var. *rhoei* from rhubarb from the United States.

Tucker (1931) found that many isolants of *P. parasitica* possess oospores agreeing with *microspora,* others with *macrospora,* and others intermediate in size. He regards the tobacco black shank organism as

P. parasitica var. *nicotianae*. He also considers *P. tabaci*, described from Formosa, by Sawada, as synonymous.

Proof of the pathogenicity of the black shank fungus has been obtained by numerous investigators. When the fungus from pure culture is inserted into the stems or buds, infection is usually evident after four to five days. If the temperature is below 20° C., it is unfavorable for the development of the disease. Temperatures of 30° to 35° C. are optimum for rapid development. The lack of a suitable temperature, no doubt, accounts for the failure of the fungus to attack seedlings in seed beds in North Carolina.

The pathogen forms two types of reproductive structures, sporangia and oospores. Several investigators have studied the emergence of the swarm spores from the sporangia, and the types and manner of fertilization (Ashby, 1928, and Krupko, 1931). The mycelium in culture is much branched and nodose, and can be grown on a wide variety of culture media. Tisdale and Kelley (1926) found that maize meal agar is especially favorable for the production of sporangia. This substratum, in the writer's studies, was also favorable for the production of oospores.

Hibernation and Dissemination.—Oospores may form in decaying leaves, but they are not necessary in maintaining the existence of the organism. Several agencies are responsible for the introduction and spread of the black shank pathogen. The following have been noted: (1) During weeding it may be spread throughout the entire seed bed. (2) Diseased plants, when set in the field, may be the means of establishing the disease in new localities. (3) The disease may first appear along drainage ditches or in situations that are flooded with drainage water from infested fields. Nolla (1928) indicated that water is an important agency in field to field spread. This is in accord with the observations of Tisdale and Kelley in Florida and of the writer in North Carolina. (4) There is evidence that infested soil may be transported on tools, farm implements, and the feet of an-

imals. Man himself in visiting diseased beds or fields may carry the organism to non-infested areas. (5) Infested soil and sporangia may be borne by the wind for considerable distances. (6) The organism may be disseminated with manure. This has long been known to be the case in the East Indies. There the soils on some plantations are enriched by applications of 16 to 32 cubic meters of *dessa mest* per bouw (1.755 acres). *Dessa mest* consists of very heterogeneous ingredients, including leaves of trees and bamboo planted along the border of the native villages, kitchen refuse, ashes, and other wastes from human habitations. These materials are allowed to accumulate in piles or in pits. *Dessa mest* is an essential manurial material on some plantations, as pointed out by Bartels (1924), since its non-application results in decrease in yield and deterioration in quality of tobacco. Since *dessa mest* is so commonly contaminated with black shank fungus, its use is attended with serious consequences. This was demonstrated by d'Angremond (1922), who inoculated tobacco with *dessa* and found that infection resulted.

Control.—In view of the fact that the black shank organism persists in the soil and that there are so many agencies for its dissemination, the prevention and control of this disease are exceedingly difficult.

The soil of seed beds should be sterilized, and special precautions should be taken to locate the beds so as to eliminate the possibility of reinfestation through drainage and flooding. D'Angremond (1920-1921) and Nolla (1928) recommend protecting the seedlings by the application of Bordeaux mixture. This should be of benefit but should be employed to supplement such sanitary precautions as sterilization of the soil, frames, and cloth. Black shank has been introduced into beds as the result of promiscuous inspection of infected seed beds. Nolla noted that the dipping of the plants from beds in which damping off occurred prevented introducing the black shank fungus into non-infested fields. Diseased seedlings, when transplanted, will sooner or later succumb,

so that scrupulous care must be exercised in selecting healthy transplants. The removal and destruction of diseased plants from fields, as soon as they are detected, have been suggested as a good practice.

The avoidance of the use of manure or compost containing diseased plants is recommended. D'Angremond showed that the disease was most serious in Java in fields and seed beds to which *dessa mest* had been applied. Thung (1932) found that disinfection of *dessa mest* with carbon disulphide is beneficial. He recommends also the burning of all diseased stalks, tops, and tobacco trash, and the disinfection of the curing-barn floors.

All of the measures that have been mentioned aim at the avoidance and amelioration of black shank. Satisfactory control seems to be possible only by the selection and breeding of resistant varieties. Such studies have been made in Java, Porto Rico, and Florida, and show that certain varieties possess a high degree of resistance. The selection of strains produced by hybridization that have this desired resistance and at the same time the proper color, texture, aroma, leaf size, and yield is exceedingly difficult of accomplishment.

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Downy Mildew or Blue Mold

INTRODUCTION.—Practically all investigations of downy mildew which have hitherto been made have been conducted in Australia, where this disease is endemic. The results of these investigations, in their most essential features, are summarized in a recent publication by Angell and Hill (1932). The studies by the writer and his colleagues of the disease as it occurs within the United States are presented in a recent report (Wolf, Dixon, McLean, and Darkis, 1934). The present account is drawn largely from these two reports, and from unpublished data of investigations made during the past year (Dixon, McLean, and Wolf, 1935).

Downy mildew first appeared in the area devoted to the culture of flue-cured tobacco in the United States, in 1931, and has continued to spread so that it now occurs ubiquitously. While it may occur on tobacco in the field in Australia, observations indicate that it is primarily a disease of seedlings both there and within the United States. Observations indicate also that the disease is of the type that may be expected to appear in epiphytotic proportions every spring, although it may vary in destructiveness.

HISTORY OF THE DISEASE.—Definite records of downy mildew on tobacco in Australia date from 1890, although it was probably present thirty or forty years earlier

(Angell and Hill, 1932). A downy mildew of tobacco was first observed in Argentina in 1888. An account of the disease embodying the investigations of Spegazzini (1898), in Argentina, was published in 1898. The first record of the occurrence of a similar disease within the United States is that by Farlow (1885), who noted it on *Nicotiana glauca,* an introduced ornamental species, near San Diego, California, in 1885. He prophetically stated at that time: "It is much to be feared that the disease which attacks *Nicotiana glauca* may sooner or later extend to cultivated tobacco. If this happens the injury to the tobacco will be very great. . . . If *N. glauca* and its parasite are once introduced into the Gulf States, the parasite might attack the tobacco grown there and then pass on to Virginia and the other states where tobacco is the most important crop." Partial fulfillment of this prophecy came in March, 1921, when the disease made its appearance in Gadsden County, Fla., and Decatur County, Ga., on seedlings of shade-grown tobacco. In their account of this outbreak, Smith and McKenney (1921) pointed out that collections of downy mildew on cultivated tobacco seedlings had been made in Texas in 1906 and that the disease may have been introduced into the Florida-Georgia area by way of California and Texas. The possible spread to Florida through gradual extension of its range is indicated by the collection of downy mildew on *Nicotiana bigelovii* in Nevada (Wilson, 1914). Burger and Parham (1921) expressed the opinion that downy mildew probably existed in Florida over a period of years prior to 1921. Smith and McKenney (1921) also suggested the possibility of its introduction with mats from the East Indies. Palm (1921) maintained that this source of introduction to the United States is improbable because (1) the mats are made in Borneo in a section where tobacco is not grown, (2) the causal organism is not known to occur in the Dutch East Indies, and (3) the chance of contamination, en route, by contact of mats with tobacco from China and India, or in storage in Holland with tobacco from Brazil and Europe, is remote.

For some unknown reason downy mildew did not again appear in epiphytotic proportions in the United States until the early spring of 1931, when it occurred throughout Florida, Georgia, and Louisiana. Before the end of the season for transplanting, it had appeared in many sections of South Carolina, North Carolina, and Virginia. During 1932 it appeared on seedlings in these last-named states considerably earlier in the season, and spread to all seed beds throughout the entire Coastal Plains and Piedmont sections of the Atlantic seaboard, as far northward as Maryland. In 1933 the disease spread to the Burley tobacco sections of western North Carolina, and of Tennessee, and extended its range as far northward as Pennsylvania.

Suscepts.—McAlpine (1901), in early investigations of downy mildew in Australia, reported that the same organism that attacks cultivated tobacco occurs also on *Nicotiana suaveolens,* a species native to Australia. Adam (1933) was able to infect *N. glauca, N. suaveolens, N. rustica,* and *N. longiflora* with the fungus from cultivated tobacco. Angell and Hill (1932) are of the opinion that all species of *Nicotiana* are probably subject to the disease because of their observations that seedlings of the following species are readily attacked: *N. angustifolia, N. accuminata, N. atropurpureum, N. bigelovii, N. calyciflora, N. chinensis, N. campanulata, N. caudigera, N. glutinosa, N. langsdorffii, N. laterrima, N. nudicaulis, N. paniculata, N. repanda, N. sylvestris,* and *N. trigonophylla.* No species appear to be immune, but some exhibit a considerable degree of resistance.

The observations in North Carolina (Wolf, Dixon, McLean, and Darkis, 1934) indicate that seedlings of all agronomic varieties of flue-cured tobacco are equally susceptible to downy mildew. In addition, they observed the disease on seedlings of tomato *(Lycopersicum esculentum),* pepper *(Capsicum annuum),* and eggplant *(Solanum melongena),* when these species were being grown near tobacco seed beds or in the same beds with diseased tobacco seedlings. Similar observations, recorded in *The*

Plant Disease Reporter (1933), have been made by a number of investigators. Potted seedlings of these three solanaceous species, when inoculated with a sporangia from diseased tobacco seedlings, became infected about a week after inoculation.

Black henbane *(Hyoscyamus niger)*, when grown in beds among diseased tobacco seedlings or when inoculated in pots under conditions favorable for infection of tobacco seedlings, remains free from attack. These observations accord with those of Angell and Hill (1932) and Adam (1933). Potato *(Solanum tuberosum)* remained free from attack when artificially inoculated. Black nightshade *(Solanum nigrum)* and horse nettle *(S. carolinense)*, two weeds of rather common occurrence in or near tobacco seed beds, are also immune to downy mildew. Other immune solanaceous species include apple of Sodom *(Solanum sodomaeum)* and Jerusalem cherry *(S. pseudocapsicum)* (Darnell-Smith, 1929).

Symptoms.—Downy mildew is primarily a seedling disease in the flue-cured-tobacco districts. It usually appears about two weeks prior to the time that the seedlings are of a size suitable for transplanting. It may, however, attack seedlings of any age, even those which are just emerging, observations which confirm those of Angell and Hill (1932).

The symptoms of the disease, its rate of spread throughout the bed, and the severity of attack are modified by such factors as weather conditions, age and vigor of the seedlings, location of the beds with reference to exposure and to soil and air drainage, density of the stand, and construction and management of the beds.

The most characteristic feature of the downy mildew disease is the presence of a dense downy coating on the lower leaf surface. The appearance of seed beds that are affected varies greatly, dependent upon the length of time that the disease has been present, the kind of weather that has prevailed, and the source of inoculum. It has been found that there are at least two sources of inoculum within the flue-cured areas; namely, sporangia

from near-by infected beds, and oospores that have hibernated in the soil of beds sown on the sites of old beds. The presence of the disease arising from sporangia as inoculum is first indicated by the general aspect of the beds. One may note, on observing a bed in which the disease is first beginning to appear, that small groups or patches of seedlings are yellowish green in color. There may be only one such patch or at most a few, even in beds several hundred square yards in area. These areas may appear in any portion of the bed, but are most commonly present around stumps or in places in which the seedlings are making a subnormal growth. Closer inspection of yellowish green seedlings reveals that the tips of many of the upper leaves droop and are limp. At this stage the fruiting parts of the causal fungus may not yet have appeared. The under surface of the lower leaves of such plants, however, may be partly or entirely covered with a downy felt. During periods favorable for the development of the causal fungus, as during cool weather when the sky is continuously overcast, the downy coating may also appear on the upper leaf surface. This is of common occurrence on leaves in thickly sown beds or on leaves that are most shaded. When, at this time, affected leaves are viewed from above, they show large irregular patches, or the leaves may be uniformly yellowish. Large, dry, irregular areas replace the yellow patches within a few hours on clear, windy days. Under these conditions the general aspect of the seed bed is as if the plants had been scalded. This scalding is most pronounced when clear weather follows a period during which every seedling has become involved. The rapid collapse of seedlings is accompanied by a characteristic odor quite like that of decaying cabbage. If oospores are the source of inoculum, there may be no evidence of discoloration of the leaves or groups of plants when the disease is first present. Instead, the disease occurs on plants standing singly or at most in small groups and at a time before they have attained sufficient size to conceal the ground. The small leaves which are usually in contact with the

soil may be cupped downward and may show no discoloration. Close examination reveals, however, that these lowest leaves are covered with the characteristic downy coating. Within two or three weeks such beds will appear like beds that have become infected from sporangia as the source of inoculum.

Usually small seedlings are killed outright, but the bud leaves of the larger ones remain apparently normal, even though all the lower leaves have collapsed. Such plants as survive may have recovered sufficiently within two weeks to be transplanted.

Pittman (1931) regards the name "blue mould" as inappropriate because the least conspicuous feature of the fungus is its blueness. When, however, fresh material is examined in early morning or when there occurs a period characterized by cold nights and rainy days, the down has a pronounced blue tint. When the lesions have dried, or after several hours of sunshine, the fructifications *en masse* are grayish to pale brown.

If, after a period favorable for infection, a period of dry weather prevails, indefinite yellow specks appear on the upper leaf surface. These are abortive lesions which may persist unchanged, or their centers may become chlorotic. The growth of the normal tissues surrounding these abortive lesions may result in the leaf surface becoming puckered or contoured. Such lesions may never bear a crop of sporangia if the weather remains dry and warm. It is for this reason that there seems to be no way of determining in advance from the appearance of any given infected leaf, whether sporangia will appear and of prophesying the date of such appearance.

During a period favorable for the development of the disease, the collapse of infected leaves usually follows, within a few hours, the production of sporangia on those leaves. In some cases, however, daily crops of sporangia may be formed along the advancing margin of a given lesion.

Seedlings that have survived an attack of downy mildew and have developed new leaves do not seem to be

FIG. 85. Leaves of seedlings affected with downy mildew or blue mold. Large dry lesions on pale green areas are evident on the upper surface, as shown by the leaf at the left. A downy grayish coating covers the underneath surface of the lesions as shown by the leaf at the right.

FIG. 86. Downy mildew on tobacco, slightly enlarged, as seen from the lower leaf surface.

readily subject to a second attack. This observation is supported by numerous observations in the field and by attempts to inoculate artificially seedlings that have recovered. No satisfactory explanation for this apparent imunity can be made at this time.

Downy mildew occurs in Australia on the leaves of mature plants and causes the formation of large lesions that may ultimately drop out (Angell and Hill, 1932). It may also appear there, during autumn, on the suckers, flower buds, flowers, and capsules. It is improbable, because of the relationship of weather conditions to the development of the causal fungus, as will be pointed out later in this report, that downy mildew will seriously attack the growing crop in the field in the flue-cured areas.

The Causal Organism

Morphology.—The fungus that causes downy mildew of tobacco has, in common with many other downy mildews, two stages in its life cycle, sporangia and oospores. The sporangia are borne at the surface of the lesions prior to the death of the tissues, and the oospores, within the dead tissues.

The sporangia are elliptical to oval bodies 15 to 28 by 12 to 18 μ, and are densely filled with pigmented, granular material and scattered oil droplets. They are borne on tree-like, dichotomously branched sporangiophores, which branch four to eight times, and terminate in curved acute apices. The sporangiophores occur either singly or in small groups. They range in height from 400 to 750 μ. The diameter of their bases is 10 to 12 μ. The length of the stalk approximates two-thirds of the height of the sporangiophore.

Sporangial formation has been studied by stripping off the epidermis and examining under a microscope. Sporangiophores arise as branches from the intercellular hyphae, and emerge from the stomates. They soon become bifurcately branched, and as development continues, a tree-like structure with blunt dichotomous tips arises. These tips enlarge into bulbous processes, and by the

time they have attained approximately half their ultimate size, a septum delimits the young sporangium from the sporangiophore. At this stage the fructifications are colorless, but due to the fact that they cover the lesions so copiously they give to the leaf surface the appearance of being covered with a white down. By the time the sporangia have attained their mature size, their color is distinctly bluish, and the sporangiophore tips have become curved and taper-pointed.

As the result of a series of all-night vigils, it was found that sporangial production is matinal. It begins with the advent of dawn, and the sporangia are mature at sunrise. Whether sporangial formation continues to take place throughout dark days is not known.

Mature oospores have been observed in material collected in five counties in North Carolina and one in Virginia. They occur within the tissues of leaves that have collapsed and remained in contact with the soil, also in leaves kept in moist chambers four or five days after the tissues have become necrotic. When such tissue is examined with low power magnification, the oospores appear as bright, reddish brown spheres embedded within the decaying leaf, and may be so abundant that a hundred or more can be seen in one microscopic field. As they become older the color becomes dark brown. Oogonia range in size from 40 to 72 μ and the oospores from 24 to 43 μ, mostly about 32 μ.

Identity.—The organism that causes downy mildew of tobacco has until recently been designated *Peronospora hyoscyami* deBy., an organism described by deBary (1863) on black henbane *(Hyoscyamus niger)*. It would appear from the observations of Angell and Hill (1932) and from the inoculation experiments of Adams (1933) and of Wolf and associates (1934) that *Hyoscyamus niger* is immune to the pathogen which causes downy mildew of tobacco. Since downy mildews are all obligate parasites that are restricted in suscept range, it seems more reasonable to regard the downy mildew of tobacco as specifically distinct from *P. hyoscyami,* a conclusion that ac-

cords with the suggestion of Adam (1933). In a previous report from this laboratory (Wolf *et al.*, 1933) it was pointed out that the downy mildew of tobacco within the United States is either an undescribed species or is identical with *Peronospora nicotianae* Speg. described, in 1891, on *Nicotiana longiflora* from collections made in Argentina by Spegazzini. While this report was in press, Adam (1933) described as a new species, which he named *Peronospora tabacina*, the downy mildew fungus, in Australia. He compared the fungus with which he was working with "authoritatively named material" of *P. nicotianae*. The following tabulations contain a comparison of the morphological features of the downy mildew fungi on tobacco:

TABLE 13

COMPARATIVE DATA ON THE MORPHOLOGY OF TOBACCO DOWNY MILDEWS

Authority	Height of sporangiophore, μ	Branching of sporophore	Size of sporangia μ	Size of oospores, μ
Spegazzini	250–500	6–7 times	18–20x 9–11	50–80
Angell and Hill	703–1024	5–8 times	13–34x10–22	28–50
Adam	500–850	5–8 times	16–29x13–19	35–60
Wolf *et al.*	400–750	4–8 times	15–28x12–18	24–43

It should be recalled that the data by Angell and Hill and those by Adam were taken from measurements of the fungus as it occurs in Australia. Adam points out, however, that the epispore of the oospores of *P. nicotianae* is regularly areolate, as his figure shows, and as was described and illustrated by Spegazzini (1891, 1898) to appear honeycomb-like, while that of *P. tabacina* is smooth or only slightly contoured. The writer has been able to verify this observation on differences in oospores, by comparison with collections made by Spegazzini in 1897 and identified by him. Not only are the oospores of *Peronospora nicotianae* areolate, but they are larger and are yellowish brown, whereas those of the downy mildew

[1] The writer has examined specimens of P. *tabacina*, from Australia, given him by L. F. Mandelson, and found them to be specifically like the downy mildew fungus in the southeastern United States.

that occurs within the United States are reddish brown to dark brown. It appears therefore that there are two distinct species of *Peronospora* that attack tobaccos. The symptoms produced by each and the morphology of the conidial stages are quite alike, but the oogonial stages are readily distinguishable. The fungus that produces the downy mildew disease of tobacco and related species within the United States is regarded as identical with *Peronospora tabacina,* which was first described, in both its conidial and oogonial stages, by Adam (1933) from Australia.[2]

Pathology of Downy Mildew.—In studying the relation of the fungus to invaded tissues and to the changes that it produces in them, use was made of stained, paraffin sections of leaves and of preparations made by stripping off the epidermis (Wolf *et al.,* 1934). Penetration is accomplished by entrance of the infection hyphae through the stomates, as was observed by Darnell-Smith (1929). The mycelium passes between the cells. Epidermal strippings show that branched haustoria enter the cells. Four to seven days after inoculation sporangiophores and sporangia are produced. Following this, the invaded cells die and rapidly collapse. In addition, tissues that are not actually invaded, together with the entire leaf or the entire plant, may collapse. This generalized effect is borne out by the experience of many growers who transplanted diseased seedlings or apparently quite normal ones from beds in which the disease was rapidly developing. When this was done, both in 1932 and 1933, usually 50 to 90 per cent of the seedlings failed to survive transplantation, unless transplanting was delayed until the seedlings had apparently recovered. It seemed that the actual loss of leaf surface, resultant from lesions, was insufficient to account for this high mortality in transplanting diseased seedlings. For this reason attempts were made to determine if toxic products are produced within

[2] While this report was in press, E. E. Clayton and J. A. Stevenson sent the writer a manuscript on the nomenclature of the downy mildew fungus in which they conclude that the fungus on tobacco within the United States is *P. tabacina.*

diseased tissues. Accordingly, a quantity of fresh leaves of diseased seedlings was macerated by grinding in a meat chopper. The crushed material was then covered with distilled water and allowed to extract for 15 hours at 38° F. The liquid portion was then removed by filtration through filter paper and placed in five small flasks. Four healthy tobacco seedlings were then removed intact from the soil and placed with their root systems immersed in the filtrate. The filtrate in the fifth flask was heated to boiling before placing a seedling in it. A filtrate from healthy seedlings was similarly prepared, and two seedlings were placed in it to serve as a control. After twenty hours the five plants in the extract from diseased seedlings were severely wilted, whereas those in the filtrate from healthy seedlings were turgid. The roots of the wilted plants were then rinsed and allowed to remain in tap water to permit recovery of turgidity. No recovery resulted, however.

In a comparable test dead, dried leaves from diseased seedlings, instead of fresh ones, were extracted in distilled water over night at 38° F. This extract also caused healthy seedlings, whose roots were immersed in it, to wither. An extract, similarly prepared, from apparently normal leaves of seedlings just beginning to recover from downy mildew, also caused healthy seedlings to wither when their roots were immersed in it. In this case, however, the wilting was less pronounced and was most noticeable on the lower leaves. These tests are interpreted to show that toxic, water-soluble substances are formed within diseased tissues and that they may permeate the entire plant. These toxic substances may account, in part, for the death of affected seedlings following transplantation.

Dissemination of Sporangia.—There are several agencies concerned in the dissemination of sporangia of downy mildew of tobacco. The spread of the disease either within an affected seed bed or from a diseased seed bed to a near-by healthy one could be readily accomplished by carriage of sporangia on the hands and cloth-

ing of laborers (Angell and Hill, 1932; Smith and McKenney, 1921). It has also been demonstrated that, in Australia, sporangia are transported by moths of the split worm, *Pthorimaea operculella* Z. (Angell, Hill, and Currie, 1930).

The fact that the sporangia are powdery and are so readily dislodged suggests that air currents should constitute the most important agency for their dissemination. Direct evidence of the transport of sporangia by air currents was obtained by Wolf and associates (1934) by means of spore traps. These traps consisted of glass plates covered with a film of glycerine that were exposed, usually to leaward, in the vicinity of diseased seed beds. After exposure the film of glycerine and adhering material were washed off, the washings were then centrifuged, and the sediment was examined with a microscope. They found that sporangia may be entrapped on these slides in varying numbers dependent upon distance of the traps from the beds and the wind velocity. They also found that sporangia were entrapped by the glandular hairs on tobacco leaves, on the corollas of *Azalea nudiflora,* on the leaves of sweet gum *(Liquidambar styraciflua),* and by the resinous young shoots of pines. Their observations also show that sporangia may be distributed within the affected seed bed or from diseased beds to healthy ones by the grower, since sporangia readily adhere to the hands of clothing.

Sources of Inoculum in Spring.—In previous investigations three sources of inoculum for the initiation of the disease in spring have been suggested: (1) old tobacco plants that survived the winter, (2) infected seed, and (3) infested soil.

In parts of Australia many plants survive the winter and, in spring, produce suckers upon which downy mildew lesions appear (Angel and Hill, 1932). While all above-ground parts of tobacco plants are killed by frosts, except in occasional years, throughout the flue-cured-tobacco areas, it is of common occurrence for suckers to arise in spring from the underground portions of stubbles

that remain alive. Downy mildew has been observed on suckers in midwinter in southern Georgia, but there is no evidence that the inoculum for seedlings comes from affected suckers in more northern tobacco-growing areas.

Angell (1929) was led to conclude that infected seed was an important source for the initiation of the disease in seed beds in Australia because of the occurrence of lesions on capsules, the presence of coenocytic mycelium in the seed from such capsules, the early appearance of downy mildew on seedlings grown from infected seed, and the occurrence of the disease on plants growing twenty miles away from the nearest plantation. Since the disease has not been seen in the flue-cured-tobacco areas on mature plants nor upon the sucker growth, there is no evidence that seed are infected in this district.

During a period of two weeks in late March and early April, 1933, isolated outbreaks of downy mildew occurred that were rather generally distributed throughout the tobacco-growing section of North Carolina. These occurred in beds located on sites occupied by tobacco seed beds during 1932.

Again, in the spring of 1934 a study was made of old bed sites as a possible source from which the disease may start. This involved a study of 106 seed beds located on old sites and 108 near-by seed beds on new sites. These beds were located in representative tobacco-growing areas throughout North Carolina. As a result, it was found that downy mildew appeared in 35 per cent of the beds on old sites from seven to nineteen days before it was evident in any of the near-by beds on new sites. Furthermore, the disease occurred in beds on old sites prior to the time that sporangia were present in the air, as shown by spore traps, in that locality. These observations clearly establish the fact that seed beds located on the sites occupied by diseased beds during the previous year are primary centers of infection for the initiation of downy mildew. There has been confusion of opinion regarding the effect of site upon the earliest outbreaks of the disease because downy mildew does not occur in all

beds sown on old sites prior to its occurrence in near-by beds on new sites. The reasons for this are not fully known, but it is reasonable to assume that it is due to such factors as differences in soil moisture, or tilth of the sites, or to the treatment given the beds between crops.

Germination of Sporangia.—The sporangia of *Peronospora tabacina* are extremely variable in their capacity for germination, just as is known to be the case with other downy mildews. Among the factors which influence germination are moisture, temperature, light, and reaction of the media. Germination does not take place except in the film of water or in an atmosphere that is saturated. Temperatures between 45° and 60° F. are most favorable for germination. Exposure to temperatures approximating 85° F. for an hour are lethal, as is exposure to direct sunlight for this period. Most vigorous germination occurs in slightly acid media.

In general, the sporangia are short lived, approximately only one per cent being viable after four hours to seven days. Angell and Hill (1932) report as an extreme case the viability of sporangia 117 days old after they had been kept in air-dry soil at a temperature of 3° to 5° C. Differences in relative humidity do not appear to be significant in modifying viability. With relatively low temperatures, 36° to 40° F., there is an increase in the length of time that sporangia remain viable, indicating the longevity is correlated with low temperature.

Angell and Hill (1931 a) found that sporangia under moist conditions, stored at 18° C., remain viable for two months, and when stored over fused calcium chloride at this temperature, retain viability for five weeks (1931 b).

Relation of Weather Conditions to Downy Mildew.—Wolf and associates (1934) were led to conclude that the cause of the downy mildew disease is closely circumscribed by weather conditions. This conclusion was based upon (1) field observations, (2) upon a study of the influence of environmental factors upon the germination and viability of sporangia, and (3) upon the maintenance of the causal fungus under partly controlled environment

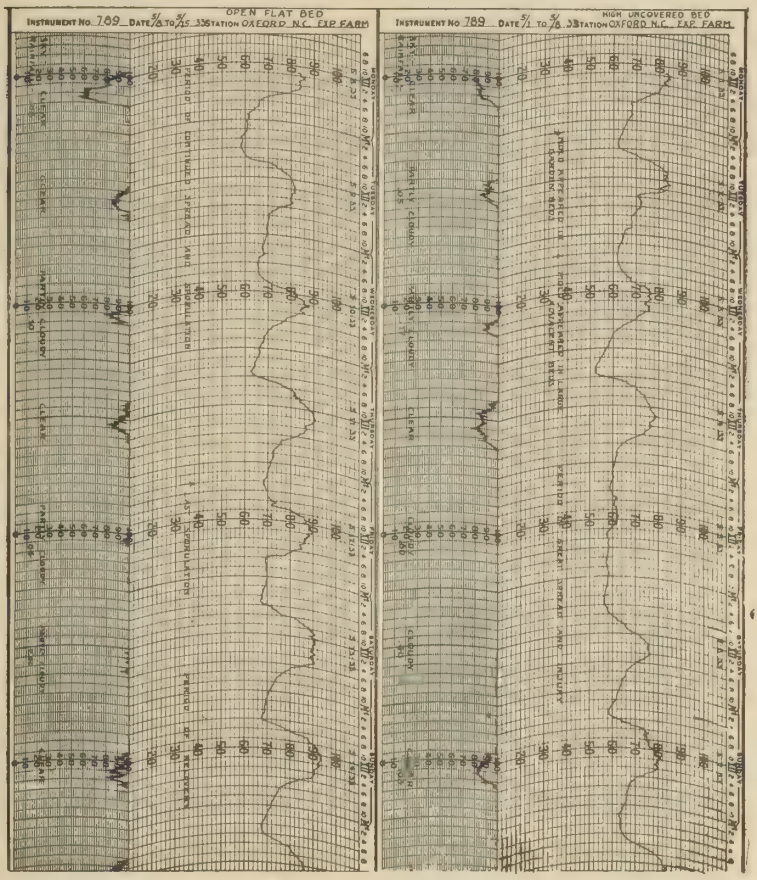
OPEN FLAT BED
INSTRUMENT No. 789 DATE 5/1 TO 5/15 STATION OXFORD N.C. EXP. FARM
HIGH UNCOVERED BED
INSTRUMENT No. 789 DATE 5/1 TO 5/15 STATION OXFORD N.C. EXP. FARM

FIG. 87. Continuous record of temperature and humidity at the Tobacco Experiment Station, Oxford, N. C., for the period from May 1 to May 15, 1933. This period covers the duration of the outbreak in the area near Oxford, N. C. The upper wavy line is the record of temperatures and the lower, that of humidities. The progress of the disease and cloudiness are also noted in the record. In further explanation it should be pointed out that a period of "blue mold weather" occurred, just prior, from the evening of April 19 to the morning of April 22. In consequence the disease began to appear in every bed in that section during the fore part of the first week in May. There then occurred another period of "blue mold weather" from the evening of May 4 until the morning of May 7, as shown by the fact that the temperature remained near 60° F. and the air remained saturated with moisture. During this period the disease involved all of the plants. In the week that followed, the weather was generally clear and warm, so that the disease was checked and the seedlings that survived recovered rapidly.

where it produced numerous successive crops of sporangia.

In general, warm sunny days with maximum day temperatures that approximate 90° F. and night temperatures that do not fall below 68° F., inhibit the development and spread of the disease. Protracted periods of cloudy, rainy weather may be appropriately called "blue mold weather." Continuous records of temperature and humidity, obtained by placing a Friez hygrothermograph in seed beds, shows that sporangia are produced in abundance, and abundant infections result when the temperatures at night fall to approximately 60° F. or below, with a relative humidity of 100 per cent, so that the seedlings are continuously wet.

Ordinarily downy mildew disappears with the advent of summer weather. When, however, tobacco seedlings were maintained under conditions of low temperature and high humidity at night, it was possible to keep the fungus alive by transferring sporangia to healthy seedlings. Under these conditions successive crops of sporangia were induced to form at will all summer long. In fact, on twenty mornings in August, 1933, crops of sporangia were developed, and subsequent crops of sporangia were produced during the fall.

Prevention and Control of the Disease.—Satisfactory prevention and control of downy mildew of tobacco has not been accomplished. Nevertheless, it is indicated from the investigations that have been made and from observations that certain practices should have a palliative influence. The fact that the disease appears in seed beds of all types of construction now employed in the flue-cured district suggests the need for experimentation on this phase of the problem. Tightly constructed beds with covers of closely woven texture stimulate the rapid and early growth of seedlings and interfere with proper ventilation. Plants grown in such beds seem to be most liable to severe injury. In order to facilitate ventilation, the employment of narrow beds and the early removal of the frames are indicated. The slight elevation of nar-

row beds above the level of surrounding soil should also be beneficial.

Sites on high ground are to be preferred to low-lying, swampy situations; sites in open fields, to those closely surrounded by woods; and beds on steep slopes, exposed to good opportunities for air drainage to those on level situations. More severe injury occurs in seed beds that are shaded during a portion of the day than in those more distant from the woods. In the selection of bed sites, therefore, attention should be given to (1) good soil drainage, (2) good ventilation, and (3) exposure to sunshine during the entire day.

Beds that are thinly sown and that contain less than half of the usual stand of seedlings suffer less damage than those that are thickly sown.

It is the usual practice to remove the seed-bed covers a few days or at most a week prior to the time for transplanting. When the covers are removed three to four weeks before the season for transplanting, and are returned at night only if frost is indicated, the seedlings are more hardy and less subject to serious damage.

The influence of temperature on sporulation and on viability of sporangia indicates experimentation on artificially heated beds. This is also indicated by the necessity of moisture for germination and infection. Such experimentation as has been done shows that if the temperatures are maintained at 70° F. or above, downy mildew may be prevented. No means of making a practical application of these findings to seed-bed practices in the flue-cured-tobacco areas have been found. Angell and Hill (1932) suggest the use of seed beds so constructed as to make possible the control of temperature and humidity.

The passage from infected areas to non-infected ones or from diseased beds to healthy ones, as during weeding, may transport the sporangia, and infection may follow. It is advisable to guard against this. Seed beds should be made in new situations each year unless the soil is steam sterilized. The use of sites occupied by beds during the previous year apparently constitutes the most

important source of primary infection in the flue-cured-tobacco areas. Beds on old sites may be expected to become infected three to four weeks prior to the general prevalence of downy mildew and one to three weeks before the disease is present in new beds. Such beds are continuous sources of sporangia that are spread by air currents to those beds that are nearest, and thence to more distant ones until eventually all seed beds are infected. Universal avoidance of old bed sites may be expected to be an effective preventive procedure. Until such time as this can be done, however, it appears to be advantageous for communities or other large areas to encourage the practice of using only new bed sites.

In some seasons observations indicate that the use of new bed sites rather than old ones may determine whether the grower will secure a sufficient number of seedlings to transplant his crop at the most favorable time. The downy mildew, occurring earlier and on smaller plants in beds on old sites, may result in thinning the stand or in checking the growth of the plants that survive so that it is late in the season before they can be transplanted. On the other hand, the seedlings in beds on new sites may be large enough to transplant before the widespread occurrence of the disease.

The destruction of all tobacco seedlings and débris which remain in the seed bed as soon as the crop is transplanted is indicated by the fact that *Peronospora tabacina* forms oospores. There is no evidence as yet that this practice would be of value.

In efforts to control downy mildew many kinds of sprays and dusts have been employed. It is practically impossible to protect adequately against infection by such measures, because of the difficulty involved in covering both the upper and lower leaf surface and in keeping them entirely covered with the spray materials. Such sprays and dusts as have been found to give some protection tend to stunt the seedlings, so that the recommendation of their use does not appear to be justified.

When the disease appears, the judicious application

of nitrate of soda should be made to stimulate the recovery of diseased seedlings. It may be unnecessary to apply nitrate of soda if an adequate supply has previously been provided.

Transplanting of seedlings from infected beds is counter-indicated. Instead of transplanting from beds in which the disease is rapidly developing, the seedlings should remain undisturbed until after recovery is advanced to the stage that new leaves and new roots have formed.

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Argentine Downy Mildew

Historical.—The downy mildew disease of tobacco that occurs in Argentina appears to be caused by an organism that is specifically distinct from the one which attacks tobacco in Australia and in the southeastern United States.[3] The following brief account has therefore been prepared, and is taken largely from publications that deal with the Argentine downy mildew of tobacco by the eminent mycologist, Spegazzini.

On December 17, 1888, Spegazzini noted this disease on the leaves of *Nicotiana longiflora* growing in a park near Buenos Aires. The disease was manifest at that time by the presence of scattered lesions having yellow borders. He found as the result of microscopic examination, when he had returned to the laboratory, that the oospores of some phycomycetous fungus were embedded within the tissues of these lesions. It was not until two years afterward, however, in the city of La Plata, that he observed the conidial stage of this organism. At that time his attention was directed to diseased plants of the same species of *Nicotiana,* growing in a moist, protected place near a building occupied by the faculty of agriculture and veterinary medicine. As a result of his observations, he prepared for publication a brief mycological note in which he described the fungus as a new species *Peronospora nicotianae* (Spegazzini, 1891). His subsequent observations showed that the fungus reappeared every year and that by 1897 the disease was generally prevalent on all plants of *N. longiflora* growing in the vicinity of La Plata. Meanwhile he became interested in a taxonomic study of wild and cultivated species of *Nicotiana,* seed of which he collected in northern Argentina, but his investigations were hampered by the appearance

[3] This conclusion accords with a report of recent studies, not yet published, by E. E. Clayton and J. A. Stevenson on the Nomenclature of the Tobacco Downy Mildew Fungus.

of downy mildew on seedlings in his nursery at La Plata. The wild species were first attacked, and thence the fungus spread to the cultivated ones, and, as a result, Spegazzini was convinced that *Peronospora nicotianae* is to be regarded as a potentially grave menace to tobacco culture. In consequence, he devoted himself to a study of the disease and published a more complete account (Spegazzini, 1898). This report includes a description of the symptoms of the disease and of the morphology of the pathogen, and contains, in addition, suggestions for prevention and control.

In a later report Spegazzini (1902) noted that *Peronospora nicotianae* was of common occurrence on *Nicotiana alpina* in the province of Mendoza, along the route between Las Cuevas and Punta del Inca. It is worthy of note, however, that this downy mildew disease did not appear in the areas devoted to the commercial culture of tobacco, and that it has never done so up to the present. As a result, no further study of the disease was made until in 1925 when Spegazzini's (1925) interest was stimulated by requests for specimens, from mycologists in other parts of the world. He and his friends made numerous efforts to collect specimens, but they were unsuccessful on account of an extended drought. He reported that the disease is found in late winter and early spring each year, but was never abundant except in 1919 when there were frequent rains and the weather was cool. Hauman (1914) and Hauman and Parodi (1921) record its presence on various species of *Nicotiana* but state that it is not found abundantly ("*Ataca diversa especias del genero* Nicotiana. *Ha sido observado sobre el tabaco en Missiones, par Spegazzini, pero parece ser muy rara,*" Hauman and Parodi, 1921).

Appearance of the Disease.—This disease on seedlings is of the necrotic type and results in their death, but on larger plants it causes the formation of leaf spots. It begins on the lower leaves and gradually involves those higher upon the stem. On larger plants it is first manifest by the presence of small, circular spots, which ex-

pand and become more or less irregular. The spots are yellow above, and their lower surface becomes covered with a grayish fuzz, the reproductive parts of the mildew fungus. At maturity such spots become dry and brittle. Oftentimes, also, the leaf surface becomes uneven with the convexity on the upper side. By the time that the tissues have become necrotic, they become grayish brown or dark brown, and may fall away, so that the leaves eventually consist of ragged, irregular fragments, and are more or less completely disintegrated.

The stems of affected plants are yellowish and have a tendency to become fasciated. Instead of growing erect, they bend on themselves, taking the form of a closed hoop, and thus resemble a diseased condition of tobacco, occurring in the province of Tucuman, known there as *jaroba* (hump). This manifestation, according to the writer's opinion, indicates that the disease is *kromnek*, a disease of virus origin described from South Africa. Spegazzini (1925) also described peculiar malformations of the stems and roots, as due to some phycomycete that he presumed to be the downy mildew fungus. The dormant buds were transformed into botryoid masses, approximately the size of a ring dove's egg, that somewhat resembled a cauliflower. Hypertrophic enlargements of the cortex of the roots were also formed. One cannot be certain, from the evidence in hand, that these stem symptoms are caused by *Peronospora nicotianae*.

Cause.—This downy mildew disease is caused by *Peronospora nicotianae* Speg. The conidial stage consists of tree-like sporangiophores that range up to 1.5 mm. in height. At the point of emergence from the leaf, the sporangiophore base is about three times as thick as the hypha from which it originates. The sporangiophore is five to eight times dichotomously branched. The tips are slender, flexuous, and 15 to 18 μ in length. The sporangia vary in size from 15 to 25 μ x 8 to 14 μ, and have a granular content. When kept in water for some time, Spegazzini (1898) states that they germinate by zoospores. The report of this type of germination is probably an

error in observation, and the motile elements were probably protozoan contaminants.

As has been shown, the sporangia and sporangiophores of *Peronospora tabacina* are variable in size, and on this basis it is difficult to distinguish it from *P. Nicotianae*. A detailed study of the two species, however, might reveal distinct differences. The oogones of *P. nicotianae* are 80 to 100 μ with colorless capsules 10 to 15 μ thick that invest the oospores, 50 to 80 μ in diameter. The oospores are dark yellowish at first and later grayish brown. Their outer membrane has a honeycomb-like design, as is shown in Spegazzini's illustration. Specimens collected and identified by Spegazzini have been examined by the writer.[4] The oospores are like those described and figured by Spegazzini. They are larger than those of *Peronospora tabacina*, are of a different color, and have very different surface markings. These characters of the oospores show that *P. nicotianae* is most certainly specifically distinct from *Peronospora tabacina* Adam.

Control.—The following measures for prevention and control were suggested by Spegazzini (1898). They are based upon his knowledge of other downy mildew diseases, and he believed that their application might reasonably be expected to result in control.

1. Investigate the origin of the seed so as to avoid using seed produced in regions infested with *Peronospora nicotianae*.

2. Immerse the seed, before sowing, in 10 per cent solution of copper sulphate.

3. Examine the fields in which it is planned to cultivate tobacco, and if wild species of *Nicotiana* occur in them, remove the plants and burn them.

Avoid planting fields in tobacco if downy mildew was present, either on wild or cultivated tobacco grown in such fields, during the previous year.

[4] From authentic specimens loaned through the courtesy of Dr. Lorenzo R. Parodi. Dr. José F. Molfino kindly sent the writer certain publications of Dr. C. Spegazzini, including his Bulletin 4, 1898.

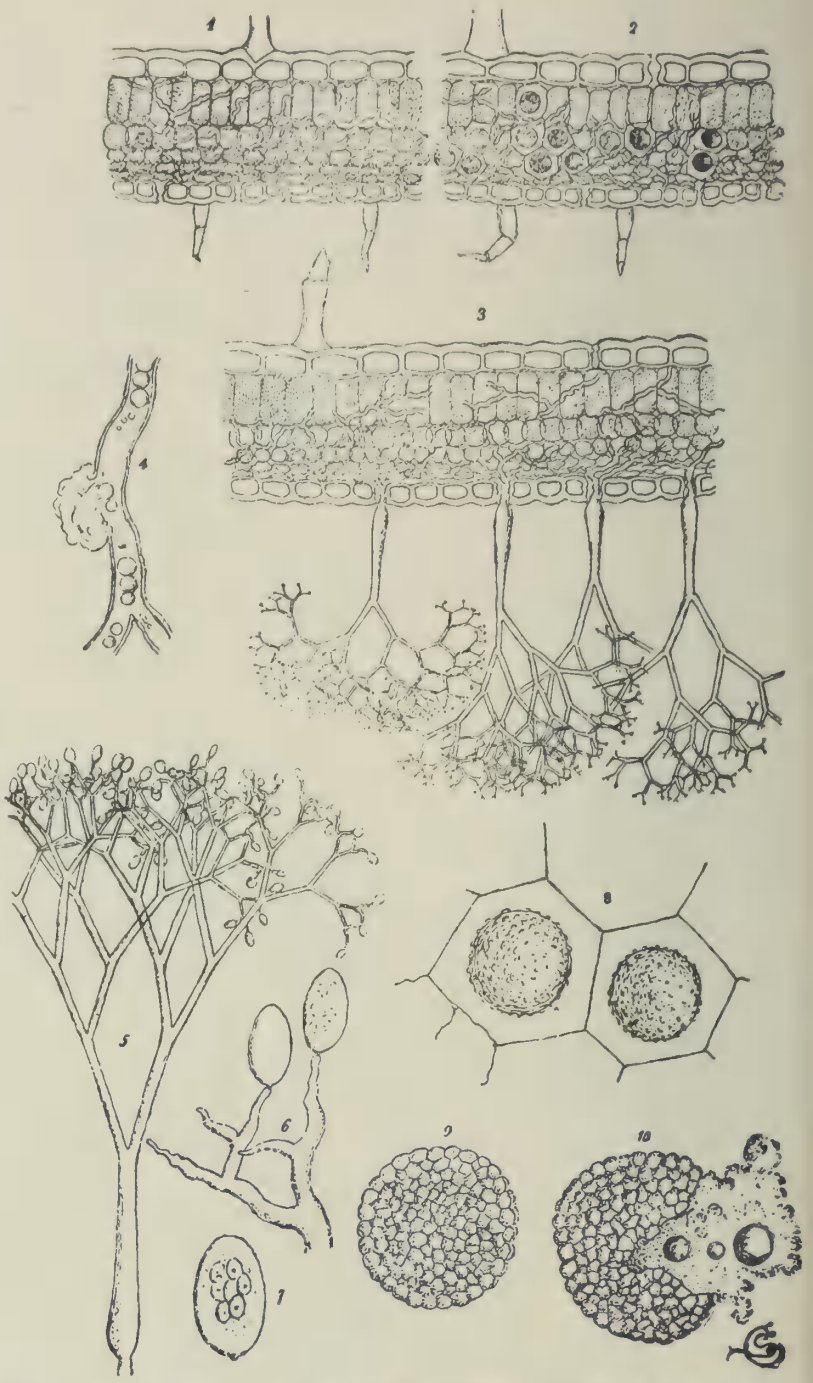

FIG. 88. Argentine downy mildew of tobacco: (1) Section of a diseased leaf showing the mycelium of the parasite. (2) Section of an affected leaf

5. Closely watch the seed beds and recently transplanted seedlings, and immediately destroy all plants that are infected. At the same time spread a layer of slaked lime over the sites from which affected plants were removed.

6. Keep the seed beds free from weeds, especially from all wild tobacco plants.

7. Sow the beds thinly, because thickly sown beds favor the disease. Avoid placing seed beds in low-lying, moist, or shaded sites.

8. Remove and burn the lower leaves because observations show that they are the first to become affected and thus to aid in propagating the pathogen.

9. The application of Bordeaux mixture or other fungicides is not advised because it would appear to be difficult to apply the fungicide to the lower leaf surface of such small plants and thus to protect them at this critical stage.

10. Mineral fertilizers rather than organic ones should be applied to stimulate the rapid growth of the seedlings and to increase their hardiness and resistance. The former type of nutrients should be of no value in stimulating the propagation of the fungus, whereas the latter type might be of aid.

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showing mycelium and oogones. (3) Transverse section of a leaf, bearing 4 sporangiophores from the lower surface. (4) Portion of intercellular hypha bearing an adhesive nodule. (5) Sporangiophore bearing sporangia. (6) Dichotomous apices with attached sporangia. (7) Single enlarged sporangium. (8) Two oogonia. (9) An oospore showing honeycomb-like outer wall. (10) A crushed oospore with its content of oil globules. (After Spegazzini.)

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Powdery Mildew

The name "powdery mildew" is always associated with a diseased condition of plants in which the affected parts are covered over with a white, cob-web-like growth that may assume at certain stages a snowy white, powdery appearance. Tobacco, in common with many other crops, is subject to a disease of this type, but it does not occur in the tobacco-growing areas of the United States. In fact, it is the only fungous disease of major importance that does not occur within the United States.

Suscepts.—The organism which causes tobacco powdery mildew occurs also on members of the *Cucurbitaceae* (pumpkins, melons, cucumbers), the *Compositae* (sunflowers, goldenrods, etc.), and certain other plants. Whether it is taken from one or the other of these suscepts species, it is indistinguishable morphologically. The pathogen consists of a number of specialized physiological strains, as shown by its inability to produce infection when used in reciprocal inoculations. Dekenback (1924), in southern Russia, successfully inoculated cucurbits with mildew from tobacco, but did not make reciprocal inoculations. Schwartz (1926) found that the fungus on tobacco in Java was identical in structure with one that involves the leaves and stems of ground cherry *(Physalis minima)*, a common weed.

Anastasia (1902) first pointed out that not all varieties of tobacco grown in Italy are equally subject to attack. He lists Virginian and Kentucky varieties as only slightly attacked under conditions in which Brazil, Big Havana, Sumatra, and Hertzgowinia tobacco are seriously affected. Smyrna and Macedonian sessile-leafed

tobacco, which are hybrids of havanensis-brasiliensis-purpurea varieties, when grown in Turkey, were observed by Ducomet (1928) to be affected.

Symptoms.—The fungus itself, present as a powdery layer over both surfaces of the leaves and upon the stems, constitutes a characteristic sign of mildew. It is first manifest as grayish white, felt-like patches upon the under side of the leaves. These patches rapidly enlarge and soon cover the entire lower leaf surface. By the time that the affected areas are plainly evident, the layer which covers them becomes powdery owing to the abundance of spores. At this stage dry brown spots may appear on the upper leaf surface. If affected leaves remain on the plants to maturity, they are thin and papery and, when cured, are entirely lacking in quality.

Names of the Disease and Its Importance.—The name "powdery mildew" or its equivalent in other languages is everywhere applied to this disease. Such German names as *Mehltau* and *Ashenkrankheit*, the Dutch

Fig. 89. Powdery mildew forming a white mealy coating over the leaves of Deli tobacco. (From Jochems.)

name *Veldschimmel* and the Javanese name *Djamoer* refer to tobacco powdery mildew.

The disease is known to occur in Java, Sumatra, Italy, Turkey, Russia, South Africa, Guatemala, Brazil, Austria, and Portugal. It is of minor importance except in Crimea, Java, Sumatra, South Africa, Japan, and Italy. It appears never to have been noted on tobacco within the United States, although strains of the pathogen are of common occurrence on cucurbits, sunflower, and golden-rod throughout the tobacco-growing areas.

No definite figures are available on the extent of the losses occasioned by powdery mildew. In some seasons and in some localities in Java and Sumatra it causes sufficient damage to warrant the application of control measures (d'Angremond, 1924).

CAUSE.—Powdery mildew of tobacco is caused by the fungus, *Erysiphe cichoracearum* DC., an obligate parasite. Among the other names by which it has been known are *Erysiphe lamprocarpa* (Wallr.) Lev., *E. lamprocarpa* var. *nicotianae* Comes, *E. cichoracearum* var. *nicotianae* Comes, *E. communis* (Wallr.) Fr., and *Oidium tabaci* Thuem. The fungus is entirely on the surface of the leaf except for the special absorbing organs, the haustoria. These penetrate the epidermal cells and absorb from them the materials used for the growth of the vegetative and reproductive parts of pathogen. There are two types of reproductive parts, conidia or summer spores, and ascospores or overwintering spores, borne in perithecia. The conidia appear in profusion during the summer to provide a means for the rapid multiplication and dissemination of the fungus. The perithecia are developed near the close of the season to aid in the survival of the fungus during winter. Ducomet (1928) found perithecia on tobacco in Turkey during August. Palm (1921) in Sumatra, and d'Angremond (1923, 1924) in the Vorstenland district in Java, and Averna-Saccá (1922) in Brazil, found only the conidial stage. The perithecial stage is apparently of rare occurrence or is entirely absent in most sections where tobacco mildew is found.

Control.—Powdery mildews have long been known to yield to control by treatment with sulphur and sulphur compounds. The most extensive studies on the control of powdery mildew of tobacco are those of d'Angremond (1923, 1924). Anastasia, in Italy, used sulphur effectually as early as 1902. Although dusting the plants with sulphur is effective, it is undesirable because of the fact that the sulphur is still present in amounts sufficient to be readily evident after the crop has been cured, and, in consequence, such leaves are of little commercial value. As a result of field trials in 1923, d'Angremond recommended the application to the soil around affected plants of 500 kg. (approximately one thousand pounds) of sulphur per H. A. (approximately 2.5 acres). This kept the crop free from mildew, whereas lime sulphur and Bordeaux mixture were ineffectual.

During the succeeding season he found with this method of application that adequate control came from the use of a much smaller amount, the equivalent of 130 pounds of sulphur dust per acre being sufficient. If the plants were grown in the shade or had three to six affected leaves at time of making the first application, this amount of sulphur was inadequate in checking the disease.

Hopkins (1930) reported complete control in Rhodesia following the application of 40 pounds of sulphur per acre, as compared with 43 to 57 per cent of mildew in his check plots. He found that, in addition to applying sulphur to the soil, one must prime off the lower affected leaves. High priming alone or sulphuring alone was not satisfactory.

D'Angremond (1923) noted that fields in low-lying situations with a high water table, and those lacking adequate light and air drainage were most subject to attack. Also fields that were closely surrounding the dessas or native villages were subject to injury from powdery mildew. These dessas were densely planted with bamboo that shut out the light and interfered with the movement of air from the near-by fields.

Ternovsky's experimentation (1934) in Crimea in-

dicates the possibilities of production of immune varieties. Strains that possess the appropriate qualities of the desired cultural type of tobacco might perhaps be developed from these immune varieties. Ternovsky found that among the 18 species of Nicotiana tested only two, *N. glauca* and *N. sylvestris*, were practically free from attack by the mildew fungus. The F_1 hybrids between *N. tabacum* X *N. glauca* and *N. tabacum* X *N. sylvestris* were attacked less severely than the *N. tabacum* parents. Furthermore, the F_1 hybrids between *N. glutinosa* X *N. tabacum*, *N. tabacum* X *N. sanderae*, and *N. rustica* X *N. tabacum*, remained entirely free from attack.

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Sore Shin or Sore Shank

Among the diseases of minor importance which probably occur every year in all tobacco-growing areas is sore shin. It has been given a number of other names including stem rot, sore shank, black shank, canker, bed rot, and damping off in the scattered published notes regarding it, which are mainly records of its occurrence. The most comprehensive study of this tobacco disease that has been made is that of Jochems (1926), in Sumatra, where the names *Rhizoctonia-ziekte* (*Rhizoctonia* disease) and *spinnewebeziekte* (spiderweb disease) are employed.

Suscepts.—A considerable number of field, truck, and ornamental plants, as well as seedlings of weeds and trees, are known to be subject to attack by the sore shin fungus. An enumeration of these is contained in two extensive papers (Duggar, 1915, and Peltier, 1916), but it is sufficient for the present purpose to note that such economic species as cotton, peanuts, alfalfa, potatoes, tomatoes, eggplant, beans, beets, soybeans, cabbage, and squash are subject to attack.

Symptoms.—As the name indicates, sore shin is a disease that appears on the stem or stalk near the soil surface. It usually first makes its appearance while the plants are still in the seed bed. A decayed area forms on the stems of seedlings, and, if conditions favor the development of the disease, the lesion enlarges until it extends deeply into one side or entirely girdles the stem, and, in consequence, the plant topples over or damps off. If in seed beds patches of dead and dying plants that are decayed at the ground level are noted, this disease may be suspected. One can not be certain of a diagnosis, however, without a microscopic examination, since species of *Pythium* can also produce a similar damping off.

If the stems are only slightly involved in decay, they may escape detection when the plants are drawn from the beds for transplanting. Such plants may survive when transplanted and may entirely recover, but more often the lesion continues to enlarge, and the plant makes a stunted growth in spite of the decayed area. By the time

the plants have reached the topping stage, a black canker extending up to the lower leaves and entirely through the cortex and wood, exposing the pith cavity in some cases, will have formed. The weakening of the stem renders such plants liable to be broken off by the wind, or the top may wither and dry through the interference of transfer of water and minerals absorbed from the soil. Usually only occasional plants in the field are affected in this manner. Sometimes, however, in low, poorly drained areas where the soil tends to remain cold long after it should have become warm, many plants may be involved.

It appears probable that infection may occur in some cases on plants in the field after they have been set. Jochems (1926) reported a strain of the pathogen in Su-

FIG. 90. Tobacco seedlings attacked by the sore shin fungus, *Rhizoctonia solani*. The stems are attacked near the level of the soil.

matra that appeared on seedlings, and another one that attacked the plants in the field.

Cause.—The cause of sore shin is a soil-inhabiting fungus, known in its sterile condition as *Rhizoctonia solani* Kühn, and in its fruiting stage as *Corticium vagum* B. and C. The sterile stage was first described in Germany in connection with a disease of potato called *scurf*. It was first reported in the United States in 1891 as the cause of a rot of sugar beets. The fruiting stage or *Corticium* stage has apparently never been found on tobacco. It was first noted on potatoes in France and was found on this same crop in the United States in 1902. The writer has observed the *Corticium* stage in small, grayish, spider-web-like patches on the surface of the soil in seed beds.

The fungus is known to be able to live on dead and decaying plants for considerable periods, and has been collected so widely on various species of plants that there

Fig. 91. Blackening and decay of the stems of mature tobacco plants near the level of the soil, caused by *Rhizoctonia solani*.

is reason to believe that it exists in most cultivated soils throughout the United States and Canada. It is known to occur, furthermore, in Japan, India, the Philippine Islands, East Indies, West Indies, Australia, and Europe, and has been noted on tobacco in all countries where this crop is grown.

The mycelium of the *Rhizoctonia* stage can be readily distinguished from that of other genera of fungi. The hyphae are large; the branches are inclined at an angle of 45° from the direction of growth of the parent hyphae and are always constricted at the point of origin from the main hypha. A septum is always present near the base of the branch. The mycelium courses through affected tissues, passing through the cells and resulting in their collapse and death.

Brown sclerotia, consisting of dense clumps of thick-walled, oftentimes barrel-shaped cells, may be formed at the surface of affected parts. The sclerotia serve to keep the organism alive during periods unfavorable for its growth.

Even though there is little evidence from controlled experiments that *Corticium vagum* is pathogenic, there remains no reasonable doubt of it from observational evidence. Jochems inoculated tobacco with a strain of the fungus isolated from purslane *(Portulacca oleracea)* with positive results. The reciprocal isolation and inoculation were also made, and infection followed. These isolants differed morphologically and culturally. Other observers have reported differences in strains of *Corticium vagum*, a matter which might be anticipated because the fungus parasitizes such a wide variety of species.

Control.—Since sore shin is essentially a seedling disease, its control centers around the production of disease-free plants for use in transplanting. Again, since the pathogen is so universally present in soils, seed beds should be sterilized each year prior to sowing the seed, regardless of whether old beds or new ones are employed. This can be done by means of heat, steam, or various chemicals, as has been previously indicated.

Seed beds that are wet for extended periods are favorable for the rapid spread of the organism and the development of the disease. Beds should be located on porous soils whose surface dries rather quickly following rains.

The beneficial effects of suitable location of the beds and of soil sterilization may be offset if attention is not given to the avoidance of partly decomposed manure in enriching the beds, for the reason that the pathogen may live saprophytically in the manure, and may be introduced with it.

The observance of these sanitary measures constitutes the primary defense measures against sore shin. Additional precautionary measures include (1) avoidance of sowing too thickly, and (2) aerating the beds by removal of the covers to promote less tender growth and to keep the soil surface as dry as possible.

Spraying of seedlings would appear to have doubtful value, although Jochems recommends the use of .03 per cent solution of methyl violet.

If affected plants are found when the seedlings are being removed from the bed for transplanting, they should be discarded.

Fields that are too wet due to poor drainage are not suitable for growing tobacco, in part because a large percentage of the plants may develop sore shin in wet lands.

Crop rotation is desirable and may be of benefit.

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SCLEROTIUM BLIGHT OR STEM ROT

A soil-inhabiting fungus of common occurrence throughout the southeastern United States is known to

attack a wide variety of wild and cultivated plants, causing them to be blighted. It does not appear anywhere to be among the major diseases of tobacco.

Suscepts.—The fungus that causes this blight is essentially omnivorous in its food habits. It has been reported on such crop plants as potato, tomato, eggplant, pepper, cucumber, watermelon, cantaloupe, squash, soybean, peanut, velvet bean, and carrots. There is no evidence in the fragmentary notes that have been published that any of the cultivated varieties of tobacco are immune nor that they possess any degree of resistance to attack. Diseased plants and those free from attack may stand close together in the same row. Tobacco may remain free from attack, moreover, in soils containing an abundance of sclerotia of the fungus.

Symptoms.—Sclerotial blight may appear on tobacco in any stage of its development. Westerdijk (1916) observed it, in Sumatra, on seedlings on which it was producing a damping-off disease. This form of sclerotial blight has not been noted within the United States, presumably because seedlings are grown at a time when the temperatures are not favorable for the causal fungus. It is usually first apparent in the southern United States during the hot days of mid-summer. Its characteristic point of attack is the stem at or near the surface of the soil. Here it forms brownish lesions that involve the cortical tissue and phloem, girdling the stem and extending both upward and downward. Gradually the xlyem tissues beneath are invaded, thus interfering with the conduction of water. In consequence, the leaves wilt, collapse, and become brown and dry. The invaded portions of the stem decay.

By the time that the disease has become well established, a characteristic sign has developed that will enable one to be certain of a field diagnosis. A copious, white, mycelial web covers over the surface of the lesions, and fan-like strands extend from the margin. Numerous spherical bodies, about the size of radish seed, occur in this web. Some of them are white, some are fawn-

FIG. 92. Sclerotium blight of mature tobacco plants. The portion of the stems beneath the surface of the soil is brown, and a cottony weft of mycelium forms just above the level of the soil. In this cottony weft are brown sclerotia about the size of radish seeds.

FIG. 93. Sclerotium blight from Japan. (From Nakata.)

colored, and some are brown, depending upon their state of maturity. These are the sclerotia of the pathogen.

No one has given special attention to the microscopic changes produced in diseased tobacco, although a careful study has been made of this same organism as it affects soybean, tomato, and sweet potato (Higgins, 1927). It acts on all of these in the same manner, and in all likelihood would attack tobacco similarly. The mycelium clings to the stems by means of peculiar holdfasts, thickened ends of hyphae. The epidermal cells and cortical cells beneath these holdfasts, to a depth of 2 to 4 cell layers, are killed before there is any evidence of hyphal penetration. The hyphae enter the dead cells through very small openings and are constricted at the point of passage. There is no evidence that penetration is accomplished by dissolution of the wall, but the puncture appears to result from mechanical pressure, because the outer wall appears pushed inward. After the death of the cells the hyphae course freely through them.

The death of the tissues in advance of penetration appears to be due to oxalic acid. This is shown by the fact that boiled and unboiled filtrates from cultures of the fungus are toxic to plants, and produce injuries very similar to those produced by dilute solutions of oxalic acid. In addition, oxalates are present in diseased epidermal cells, in the hyphae of the pathogen, and on the hyphal surfaces.

Names of the Disease and Its Range.—The disease has commonly been called "Southern blight," because of the wide suscept range of the pathogen in the southern United States. Sclerotium blight or sclerotial blight is to be preferred because it indicates the nature of the casual organism. Westerdijk (1916) used the designation *sclerotien-ziekte* (sclerotial disease) for the disease as it occurs on tobacco in Sumatra.

Outside of the United States the disease is known to occur in Sumatra, the Philippine Islands, Japan, Africa, and the West Indies. It does not occur in the tobacco-growing areas of the northern United States, probably

on account of the great susceptibility of the fungus to freezing.

Cause.—In the Annual Report of the Florida Agricultural Experiment Station for the year 1893, Rolfs described the casual organism as *Sclerotium* sp. It was not given a binomial until 1911, when Saccardo used the name *Sclerotium Rolfsii* in honor of Rolfs who first communicated specimens to him. *S. Rolfsii* is one of the sterile fungi whose usual means of propagating itself is by sclerotia. These structures are spherical bodies 500 to 800 μ in diameter, and they consist of fungous parenchyma throughout, with a rind of thick-walled cells. The distinguishing feature of the young hyphae is that they possess clamp connections, characteristic of *Basidiomycetes*. Nakata (1926) maintains that the basidial stage occurs in Japan and has been identified as *Hypochnus centrifugus* (Lév.) Tul.

No one appears to have attempted to inoculate tobacco with *Sclerotium Rolfsii*. The inoculation experiments of those who have studied the disease of sweet potatoes, velvet beans, soybeans, tomatoes, and peanuts, caused by this fungus, clearly demonstrate that it is pathogenic to these several suscepts. Young plants exposed to high soil temperatures and growing in soils with a high moisture content are more subject to attack than more mature plants.

Westerdijk claims that the sclerotia remain viable in the soil for six to seven years. Manifestly the sclerotia are the means by which the fungus hibernates and by which it is carried over periods when the available food supply is limited. This is shown by the growth in culture of sclerotia that have been kept dry for two years. Westerdijk states that in Sumatra it attacks *Crotolaria*, which is of common occurrence in tobacco fields, and that it spreads from this plant to tobacco.

Environmental Factors.—The optimum temperature for *Sclerotium Rolfsii* is between 30° and 35° C., and no doubt this is the principal factor in determining the geographic range of the fungus and the severity of the

disease (Higgins, 1927). In areas where the mean summer temperature is about 20° C. sclerotium blight does not occur on tobacco. During protracted hot periods in North Carolina the disease has been noted to destroy plants in areas as large as a quarter-acre in extent.

Under controlled conditions it has been found that the fungus does not thrive in alkaline soils. Since tobacco thrives best in acid soils, and since it is difficult to maintain an alkaline reaction in soils devoted to tobacco growing, this observation does not offer a feasible means of control of sclerotium blight.

Control.—No definite measures of control of this disease have been devised. There is little likelihood that any benefit will result in its control from any system of rotation, because the casual fungus is pathogenic on such a wide variety of plants. The problem of control should be investigated thoroughly, especially as regards whether varietal resistance occurs.

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Fusarium Wilt of Tobacco

Many cultivated crops are subject to Fusarium wilt diseases, some of which are very destructive. Fusarium wilt of tobacco has many features in common with this type of disease of other crops, but it is not known to be a disease of major importance in any tobacco-producing section.

Suscepts.—In addition to Maryland Broadleaf, upon which the disease was first noted in Maryland in 1916 (Johnson, 1921), it has been found also on White Burley in Ohio and Kentucky. Johnson also inoculated *Nicotiana glauca* and *N. rustica*, both of which species became infected. Potatoes, too, were used by him in inoculation tests with the tobacco wilt fungus from several

sources. He used several strains of the potato wilt organism, *Fusarium oxysporum* (Schlecht.) Wr. to inoculate tobacco. The results of these inoculations showed that the tobacco wilt organism can produce infection of potatoes and the potato wilt fungus can infect tobacco. The symptoms are not identical, however, nor are the organisms from the two suscept species morphologically and physiologically alike, as will be discussed more fully in a succeeding paragraph.

Symptoms.—Evidently plants of any age are subject to attack. Young plants in the field may exhibit no external evidence of disease unless favored by a relatively high soil temperature, which does not normally obtain in the United States until the plants are quite mature. The earliest symptoms on large plants are a wilting, yellowing, and finally death, but not decay of the leaves. Sometimes the leaves on one side of the plant only are affected, and in other cases the complete collapse of all the leaves may occur. Moore (1926) noted the occurrence of one-sided attack in South Africa, and that the leaves were dwarfed and yellow on the affected side. The top of affected plants was usually bent over. This accounts for the name *kromnek-ziekte* (crooked neck disease) in designating this disease in South Africa.

If the roots of wilted plants are examined, a portion of the small ones only, or the large ones also, may be found to be dead. The external appearance of affected plants so closely resembles that of plants attacked by the Granville wilt organism, *Bacterium solanacearum*, that it is difficult to make a satisfactory diagnosis in the field. If, however, one makes a section of the root, stem, or midrib, the xylem portion of the vascular system, as in the case of bacterial wilt, will be found to be brown or blackened. If pressure is applied, no "ooze" will appear at the cut end, as characterizes the decay caused by bacterial wilt, and instead the surface of the several vessels will remain dry.

The Fusarium wilt fungus does not appear to be able to cause the decay of the cortical portions nor of the

parenchymatous tissues. It is confined to the vessels which it invades more or less completely, as evidenced by the proportion of wilted and dead leaves. The vessels become clogged with mycelium, but toxic substances rather than mechanical occlusion are believed to be the cause of death of the plants.

History and Range of the Disease.—Fusarium wilt was first definitely recognized (Johnson, 1921) in 1916, when it was observed to be present on 10 to 20 per cent of the plants in a field of six acres. The following year it was observed in two additional fields in Maryland, in 1919 in a field in Ohio, and in 1920 in Kentucky. Its range is little known because of the likelihood of its confusion with bacterial wilt or even with black root rot. It may be identical with wilt diseases noted in other por-

Fig. 94. Fusarium wilt of Maryland tobacco. The leaves on one side of the plant are wilted, while those on the opposite side are turgid. (From James Johnson.)

tions of the world. Lounsbury (1906) reported a wilt of tobacco in the Kat River Valley, Cape of Good Hope, whose cause was not determined, and, to judge from the description, may have been the Fusarium wilt disease. Moore (1926) later identified a wilt disease in this same valley as the Fusarium wilt and stated that it had been a serious menace there for twenty years. In a more recent account (Moore, 1933) she regards the disease, called "wilt" by Lounsbury, as due not to a species of Fusarium but to a virus.

Petch (1909) noted, in 1907, that tobacco in Ceylon ripened prematurely and in patches. He isolated a Fusarium from such plants that may have been the pathogen. This can not be known since species of Fusarium are universally present in decaying roots and in soils. It has been reported, in addition, in Canada, the Philippines, Florida, and North Carolina.

Delacroix (1906) ascribed a disease of tobacco in France to a species which he named *Fusarium tabacivorum*. It is now impossible to identify from his description either the disease or the causal organism.

Cause.—This wilt disease of tobacco is caused by an imperfect, soil-inhabiting fungus, *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae* Johnson, that resembles closely the potato wilt Fusarium. It forms two types of conidia, one-celled microconidia, and 3- to 5-septate macroconidia, that are salmon colored *en masse* and are somewhat larger than those of the potato wilt fungus. This difference, together with differences in cultural growth and in the results of reciprocal inoculations as have been mentioned, leads to the conclusion that the Fusarium on tobacco and the one on potato are distinct.

The cultures used in the inoculation experiments (Johnson, 1921) were obtained by planting pieces of diseased stems on acidulated potato agar plates. Subcultures were made from the colonies that developed on these plates. The conidia and mycelium from cultures thus obtained were placed in the soil near the wounded stems, or else sterilized soil was mixed with the inoculum.

Seed were then sown in the contaminated soil, or seedlings were transplanted into it. The percentage of infection was greatest when the young plants were set in infested soil. A summary of the studies on pathogenicity shows that the following conditions are necessary for a high percentage of infection and the favorable progress of the disease: (1) heavy infestation of the soil, (2) wounded stem tissues, (3) a high relative humidity, and (4) a susceptible variety (Johnson, 1921).

Evidence from field observations indicates that hot, dry seasons favor the Fusarium wilt. This is supported by the results of trials with "control temperature tanks" (Johnson, 1921), in which a range of temperature from 28° to 31° C. was found to be optimum for the development of the disease, and with lower soil temperatures there was a correlated decrease in severity.

Control.—There is no experimental evidence upon which to base recommendations for the control of this disease. Certain measures, however, are known to be effective with diseases of a similar nature on other crops. These include the following:

1. Infested fields should not be planted with tobacco in successive seasons, since the causal fungus lives in the soil from year to year. The duration of the period of survival of the tobacco Fusarium is not known.

2. In all likelihood new fields become infested from the use of diseased transplants. This can be avoided by the selection of new sites for the seed beds that should be so located as to be free from surface drainage and from refuse from infested fields.

3. Varieties of tobacco are known to differ in resistance to Fusarium wilt. White Burley which is resistant to black root rot is reported to be resistant to this wilt. If the disease should become sufficiently severe to warrant selection of resistant strains, it appears feasible that resistant strains of other commercial types could be developed. Moore (1926) observed that Joiner tobacco exhibited resistance in the Transvaal. Shade-grown Cuban tobacco and a Wisconsin binder selection are quite

resistant to wilt. Pryor and Orinoco types are also relatively resistant.

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SCLEROTINIA DISEASE

Among the diseases that are of little economic importance outside of Europe is one to which the name "*Sclerotinia* disease of tobacco" may be given. The fungus which causes it is soil-inhabiting. Diseases of other crops caused by species of *Sclerotinia* are prevalent in regions with cool climates, and in warmer sections species of *Sclerotinia* attack such crops as are grown in early spring or in late fall. The disease, as it occurs on tobacco, has been given special consideration by Oudemans and Koning (1903) in Holland and by Böning (1928) in Germany. It occurs also in Japan. The writer first noted the disease on seedlings in North Carolina in 1933, and Clinton has reported its presence on seedlings in Connecticut. Within the United States it does not appear to involve mature plants in the field.

SUSCEPTS.—The fungus that causes the *Sclerotinia* disease of tobacco attacks this crop at any stage of its development ranging from seedlings to mature plants, and even causes a decay of plants that have been harvested and hung in barns to be air-cured. Böning observed it also on *Nicotiana rustica*. Besides, it is known to parasitize such crops as beets, cabbage, beans, carrots, tomatoes, potatoes, celery, lettuce, and sunflowers, on some of which it causes serious losses.

SYMPTOMS.—The stems of seedlings in the seed bed are attacked at the ground level. The disease is manifest

by the occurrence of plants in small groups that are either wilted or have been rotted off at the surface of the soil. Closer examination reveals that the stems are involved in a soft rot, and that both the decayed area and the soil around the affected stem are covered with a dense, white weft of mycelium. In this weft may be embedded elongated, hard, black bodies, the sclerotia, about one-sixteenth to one-eighth of an inch long.

Large plants in the field that are attacked present an appearance similar to affected seedlings. The affected tissues are brown, and the cortex is decayed, with strands of phloem fibres left intact. If the stem is split open, the pith is found to be decayed, a white weft fills the pith cavity, and sclerotia are embedded in this weft.

When the harvested plants are hung in the curing barns, there may appear on the midrib and principal veins a condition known as *Fäule* (rot), *Dachfäule* (house rot), or *Dachbrand* (house burn) (Behrens, 1893). This decay may have its origin while the plants are still in the field. The decay of the midvein extends to the laminar tissue as the mycelium is extended in its growth, or is spread from one leaf to another if the leaves are hung in contact.

Cause.—This disease is caused by one of the disc fungi, *Sclerotinia libertiana* Fkl. (*Sclerotinia sclerotiorum* (Lib.) de By.). It possesses two stages in its cycle of development, sclerotia and apothecia. The sclerotia are masses of densely compacted, vegetative parts by means of which the fungus lives over unfavorable periods. They constitute the dormant stage. These sclerotia germinate in spring to form the apothecia by developing a few to a dozen dark brown stalks, whose length depends upon the depth to which the sclerotia are buried in the soil. These stalks expand above into saucer-shaped discs, 4 to 8 mm. across, whose inner surface is lined with club-shaped sacs each containing 8 hyaline, elliptical spores. When these spores are germinated, they again form mycelium and eventually sclerotia. If the spores are grown in culture, the mycelium bears mi-

croconidia whose function is not understood, but there is evidence that they are spermatia, male reproductive elements.

Oudemans and Koning (1903) described as a new species *Sclerotinia nicotianae*, which they investigated in connection with the *Sclerotinia* disease in Holland, and they proved its pathogenicity. Apparently their fungus is identical with *Sclerotinia libertiana*, which is known to be widely distributed throughout the world and to parasitize a wide variety of plants.

Control.—The control measures that are recommended by Böning include:

1. The selection of sites for seed beds that are known to be free of sclerotia, and of places where tobacco has not been grown for a period of years.

2. If permanent seed beds are employed, affected plants should be removed as soon as they are found, and the surrounding soil should be soaked with formaldehyde, 1 to 100 solution.

3. Set only healthy seedlings. If the plants in the seed bed are affected, the pathogen will most certainly be carried to the field at transplanting.

4. Seed beds should be properly aerated, watered, and drained, because the fungus is favored by humid conditions.

5. Fields in which the losses have been serious should not be returned to tobacco for a period of years.

6. The stubbles of affected plants should be pulled and burned after harvest. The fields should then be plowed to bury the sclerotia so deeply that apothecia can not form.

7. Avoid harvesting in wet weather or while the plants are laden with dew or rain.

8. Avoid mixing leaves from healthy and diseased plants. Harvest them separately.

9. Hang the tobacco so as to provide proper aeration, and in houses so constructed as to permit the free circulation of air.

10. By early planting and proper fertilization, have

the crop mature in the field before danger of frost appears.

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FROG EYE

A leaf spot disease of tobacco that is cosmopolitan in distribution occurs in all tobacco-producing areas within the United States, and has been reported from Mexico, Cuba, Porto Rico, Trinidad, Brazil, Dominican Republic, Austria, Germany, Java, Sumatra, Ceylon, Malaya, Japan, Mauritius, Nyasaland, Philippine Islands, Rhodesia, and Australia. In some seasons it is of no consequence, whereas in others it is the cause of rather serious damage. Much of our knowledge of frog eye comes from the investigations of Jochems (1931) and Meurs (1932) in Sumatra, and of Hopkins (1929, 1933) in South Africa. Jochems states that it has been present in Sumatra ever since the introduction of tobacco culture, but was of no importance during the period from 1910 to 1929. In 1930, 1931, and 1932, however, it occurred in serious proportions. Likewise it appears to have occasioned considerable losses in South Africa and Australia within the past few years.

SYMPTOMS.—Frog eye may appear on seedlings, on plants in the field, and on the harvested crop. The distinguishing feature of this disease, in the mature condition, is the presence of ashen-gray spots with narrow, brown to black, raised borders. The spots are mostly circular in outline, although they are sometimes somewhat angular, and are 1 to 10 mm. in diameter. Young spots may be tan-colored but become gray when the fructifications of the fungus appear.

In North Carolina frog eye has been noted on seedlings ten to fourteen days old. The lesions may then

FIG. 95. Frog eye leaf spot on mature flue-cured tobacco and on a late-sown seedling leaf. The spots are grayish with dark elevated borders.

occupy all or most of the leaf surface of the primary leaves, and the death of the seedlings results. The lesions on seedlings are brown, and they may not become grayish until the fructifications of the pathogen appear. The early occurrence of the disease on the seedlings indicates that the disease is seed-borne.

In wet seasons infections that are incipient on the crop when it is harvested, continue to develop after the leaves have been hung in the curing barns. Hopkins (1929) and Jochems (1931) regard this phase of the disease as more destructive than that on plants in the field. During curing, bluish black to pale bluish gray spots develop, which are less sharply delimited than those on the growing leaves. This condition is called "pole sweat" or "pole burn" in Rhodesia (Hopkins, 1929) and is favored by excessively high humidities during the early stages of curing, by rainy weather during harvest, and by delay in harvesting until the leaves are over-ripe. Jochems (1931) states that the spots on leaves of superior quality largely disappear when the cured product is undergoing fermentation prior to its manufacture. Leaves of poor quality, however, are rendered unmarketable when spotting develops during curing.

Names of the Disease.—The name "frog eye" is the most commonly used designation for this disease wherever English is spoken. Vincens (1918) employs a name which means leaf burn. In the Dutch East Indies *spikkel* (spot) is preferred, but such other names as *roode roest* and *loodsvleken* are applied. The Javanese terms for this disease are *patik abong, patik poetih,* or *patik bolong.*

Cause.—Frog eye is caused by the fungus, *Cercospora nicotianae*, first described by Ellis and Everhart (1893), from material collected in North Carolina. This name is synonymous with *C. raceborskii* Sacc. et Syd. (Sacc. Syll. Fung. 10:1070), collected in Java, and described as distinct because the conidia are larger than those of *C. nicotianae.* The frog eye organism possesses conidia which vary greatly in size and cover the range

60-180 by 4-4.5 μ given for *C. raceborskii*. Another species, *C. solanicola*, has been reported to occur on tobacco in Brazil.

CONTROL.—Evidence obtained from experiments on the effects of seed treatment indicate that the frog eye organism is seed-borne. This shows that its control centers primarily upon the production of healthy seedlings, and that the seed should be disinfected with such chemicals as formaldehyde or silver nitrate prior to sowing.

Hopkins (1929) noted that the disease was less severe in Rhodesia on seedlings that were liberally supplied with fertilizer. If it appears in the seed beds, he recommends spraying the seedlings with Bordeaux mixture (4-4-50). The disease may be kept in check in the fields by priming off the lower leaves and not permitting the leaves to become over-ripe before they are primed.

In the barns any method of management that will favor the rapid drying of the leaves will prevent spotting. This is, of course, not a difficult matter with flue-cured tobacco. With other types of curing, special efforts must be directed toward securing the rapid drying of the leaves in the early stages of curing. This is possible only if the barns are ventilated in such a way as to permit the moisture to escape rapidly. It may be necessary to apply artificial heat to accomplish this. The making of several small fires, so located as to facilitate uniform heating of the barn, will hasten drying. The ventilators at the roof should be open meanwhile, and those at the bottom and the sides should be closed.

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BROWN SPOT OF TOBACCO

Brown spot, also called "red rust" in some tobacco-growing areas, may be regarded as one of the diseases of tobacco that is usually of minor importance but may at times cause considerable damage. Recent studies by Tisdale and Wadkins (1913) summarize the status of our present knowledge of this disease. It appears to have been first recorded from North Carolina by Ellis and Everhart (1892) in a brief mycological account. Hopkins (1932) reports it as a disease of minor importance on flue-cured tobacco in Rhodesia.

SYMPTOMS.—Lesions appear on the leaf blades and petioles and on the stalks. Those on the blades are at first small translucent areas of circular outline, unless bordered by the larger veins. As the spots enlarge, the centers die and become brown, leaving a sharp line of demarcation between diseased and healthy tissue, especially during dry weather. During rainy periods, however, there may be a yellowish halo on the advancing margin of the lesion, and the spots may attain a diameter of 1.5 to 2.5 cm., with concentric markings. If the spots are numerous, they fuse and may thus render the entire leaf worthless.

Sunken, elongated lesions appear on the stalks and petioles. The stalks may be girdled by the numerous lesions that involve the cortical tissue and rarely extend to the cambium.

SUSCEPTS.—Observations made in the field and inoculation experiments indicate that tobacco alone is subject to attack. Potatoes, tomatoes, and peppers grown in pots remain free from infection when inoculated with the causal organism.

Tisdale and Wadkins (1931) noted little difference in varietal resistance among the following varieties: Adcock, Big Stem Orinoco, Cash, Bonanza, Crutcher, Improved Warne, White Stem Orinoco, McAdoo, Yellow Pryor, and Narrow leaf Orinoco. Little or no infection developed

FIG. 96. The brown spot disease caused by *Alternaria longipes*. (From Tisdale.)

FIG. 97. Spotting of tobacco caused by *Alternaria* sp., in Japan. (From Nakata.)

on vigorous seedlings with 4 to 6 leaves when atomized with watery suspensions of conidia. If the plants were stunted, however, infection was readily secured at any stage of growth when this method of applying the inoculum was used. They employed plants 14 to 16 inches high for many of their inoculation trials, and infection was evident five to eight days after inoculation.

Cause.—Brown spot is caused by the fungus *Alternaria longipes* (E. and E.) Tisd. and Wadk. The organism was first described, in 1892, from material collected in North Carolina, and was at that time given the name *Macrosporium longipes* E. and E. (Ellis and Everhart, 1892). It forms chains of brown, clavate, septate conidia borne on small fascicles of conidiophores which project from either leaf surface. These conidia germinate if a film of moisture is present, and within twelve hours the infection-hyphae will have penetrated the epidermal cells directly or will have entered through the stomata. The mycelium passes both between the cells and through them causing the collapse and death of invaded tissues. In the course of time conidia are formed at the surface of the lesions and may serve for the further propagation of the pathogen.

The organism overwinters on old stalks left standing in the field or lying on the surface of the ground, as evidenced by the fact that conidia are present on the surface of the lesions, and that it has been isolated at various times during the winter and spring. In all likelihood the decay of the stalks that have been ploughed under in the fall interferes with its survival.

Hopkins (1931) recognized that the organism should be regarded as an *Alternaria* rather than a *Macrosporium* and so used the name *Alternaria longipes* (E. and E.) Mason.

Infection is influenced by conditions of temperature and humidity. Inoculations made at seasons when the temperature was below 19° C. resulted in no infection. When the mean temperature varied from 26.5° to 31° C. severe infection followed; medium infections were se-

cured at temperatures of 23° to 25° C. A relative humidity of 90 per cent, which was maintained in the pathogenicity studies, favors the rapid extension of the lesions, whereas under less humid conditions the spots tend to remain smaller.

Control.—The studies on this disease indicate that its control should be accomplished as the result of turning the stalks under as early as possible after the crop has been harvested. This procedure should facilitate the early decay of the suscept tissues and thus reduce to a minimum the chances of survival of the pathogen in the refuse. This operation should be supplemented by crop rotation, in part to escape primary infection from survival in refuse from a previous crop and in part to avoid root knot, which has been observed to influence the presence of brown spot through hastened maturity and insufficient water supply.

Proper cultivation and fertilization are advisable to promote vigorous growth. Furthermore, leaves that have reached the proper stage of maturity, and which are more subject to attack than vigorously growing leaves, should be primed so as to prevent the spread of the pathogen successively to the leaves above.

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Gray Mold of Tobacco

The gray molds caused by species of *Botrytis* are of common occurrence on fading flowers of a considerable number of cultivated crops and ornamental plants. They are usually prevalent in the cooler seasons or in portions of the world where the climate is cool. Gray molds also are the cause of decay of a variety of vegetables and

fruits in storage or in transit to market, and as such are of great economic importance.

The gray mold disease of tobacco is characterized by a gray, moldy coating that covers all affected parts. It is not to be regarded as a disease of major importance to tobacco. No extensive investigations of this disease have been made, but a recent report by Wolf (1931) embodies our knowledge of the present status of the problem.

The Disease

History, Distribution, and Importance.—Fragmentary notes, aside from the above-mentioned account, are the only published statements of *Botrytis* as being pathogenic to tobacco. The mention by Sorauer (1886) of *Botrytis* on seedlings in seed beds in Germany appears to constitute the first account of this disease. DeToni (1893), nine years later, noted a leaf spot in Italy, the cause of which he identified as *Botrytis vulgaris* Fr.

In compilation dealing with diseases of tobacco Peters (1912) mentions the occurrence of *Botrytis cinerea* Pers. in Java. He states that the stems of seedlings near the surface of the soil are involved in decay. Their roots and those of older plants become decayed, and large spots appear on the leaves. Ultimately the capsules are attacked, from which it is inferred that the fungus may be seed-borne. He notes, furthermore, that it may appear on the cured leaves.

Pape (1921) and Böning (1928) observed *Botrytis* on flowers of tobacco in Germany. Diseased corollas, that, in falling, chanced to lodge on leaves, served as inoculum for leaf spots. Wolf (1931) is of the opinion that gray mold has existed as a seedling disease in North Carolina for years. It was present in practically all seed beds seen, several hundred, during 1928 and 1929. In a few cases it was so abundant and so destructive that no plants were drawn from the beds for transplanting. Growers in some cases attributed the mortality of plants set in the field to the fact that these plants bore gray mold lesions at the time they were removed from the beds.

FIG. 98. Gray mold on stems of tobacco plants. Plant A, girdled by the fusion of lesions that originated at the bases of petioles. Plant B bears lesions at the nodes. Plant C shows an elongated lesion that has girdled the stem and caused the death of the top. Plants D and E indicate that the gray mold fungus passes from blade through the petiole and into the stem.

Gray mold, in addition to the regions previously mentioned, is known to occur on tobacco in Guatemala, Russia, and Japan.

Symptoms.—Gray mold, in the flue-cured-tobacco districts, is essentially a disease of seedlings and appears about the time the tobacco plants are of sufficient size to be transplanted. It sometimes continues to develop throughout the remainder of the season on plants that are left in the beds after the close of the season for transplanting. Affected plants are, of course, usually discarded at the time of removal from the seed beds. If they are overlooked and chance to be transplanted in the field during a rainy period, they may perish; on the other hand, if planted in a loose, moist soil during a relatively dry period, the larger proportion will completely recover.

The disease first appears on the lowermost leaves, during relatively dry weather, as large, brown, dry spots, bordered by a yellowish zone. During rainy periods wet rot lesions are formed, and the surface of the invaded tissues is covered with a grayish coating of conidiophores and conidia. This coating constitutes a characteristic sign of the disease, and is infallible as a criterion for diagnosis in the field. In favorable weather the blades are soon completely invaded and become dry, though remaining attached. Meanwhile the fungus has grown downward through the petiole into the stem. The resulting stem lesions are sunken brown to black. The size of these lesions appears to be governed by moisture conditions. In dry weather they remain small, and the wound soon heals. In wet weather, on the contrary, the lesions extend around the stem, and the top is killed in consequence of this girdling. The blackened areas on the stems may become several inches long and may be covered with a dense grayish coating like that on the affected leaves. If the leaves become detached, and no conidiophores and conidia are present, the stem lesions may be confused with those produced by the sore shin fungus, *Rhizoctonia solani*. In seed beds abandoned because of the occurrence

FIG. 99. Gray mold of tobacco as it appears during rainy weather. The profuse growth of conidia and conidiophores was induced by placing the leaves and stems in a moist chamber for twenty-four hours. A dense, white, mycelial weft formed at the notch a cut in the stem at the margin of a lesion.

of the disease, all of the lower leaves generally become invaded and dry. Leaves higher on the stem bear large dead patches.

Microscopic examination of invaded tissues shows that the mycelium of the pathogen is intercellular. The hyphae force their way between the cells as a result of their ability to produce a dissolution of the middle lamella. The cells behind the zone that is being invaded collapse and dry rather quickly. All tissues of the leaf and petiole may be invaded.

Cause.—Gray mold of tobacco is caused by a fungus that the writer tentatively identified as *Botrytis cinera* Pers. (Wolf, 1931), because the original description of this fungus is too inadequate to make identification certain. The observations of Peters (1912) on the occurrence of *Botrytis cinerea* as a parasite of tobacco seedlings and as a saprophyte on cured leaves, and those of Pape (1921) and Böning (1928) on its presence as a semiparasite on fading flowers, indicate the possibility of the same species being concerned in the stem rot, pole burn, or house burn of tobacco investigated by Sturgis (1892) in Connecticut, Virginia, and Kentucky. Sturgis noted this decay on tobacco cut during warm, foggy weather and hung in curing barns, and identified the primary cause as *Botrytis longibrachiata* Oud.

Under the name *Dachbrand* or *Rippenfäule,* Behrens (1892) described a decay of tobacco in curing barns in Germany, presumably identical with the stem rot described by Sturgis. Behrens was of the opinion that the causal organism is *Botrytis cinerea* of which *B. longibrachiata* was merely a strain or form. Sturgis (1893), in a later report, maintains that *B. cinerea* and *B. longibrachiata* are easily separable because of differences in habit of growth, in color, and in size of reproductive structures. Whether these two are identical and whether *B. vulgaris* is also identical remain to be determined.

Environmental Factors.—Observations indicate (Wolf, 1931) that the epiphytotics of gray mold of flue-cured tobacco in 1928 and 1929 were correlated with abun-

dant rainfall and a large proportion of days when the maximum temperature was 77° F. or below, conditions optimum for the growth of the pathogen. In 1930, when gray mold was scarce, the season was abnormally dry, and there was a relatively large number of days when the maximum temperatures were such as to impede or inhibit the growth of the pathogen.

CONTROL.—No one has conducted experiments to determine the means of prevention and control of gray mold. The fact that the disease is not equally destructive, in a given season, in all seed beds may be of significance. Those beds situated on low-lying ground that is poorly drained and that, in consequence, remains wet during seasons of normal rainfall may be seriously damaged by gray mold. This condition is further aggravated if proper air drainage is prevented by a dense growth of forest surrounding the seed beds. If, however, the beds are located on well-drained slopes in situations where the movement of air currents is unimpeded, there is little damage from gray mold. The proper selection of seed-bed sites is apparently of primary importance in preventing this disease.

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Olpidium Seedling Blight

There has been noted on tobacco seedlings in the seed beds in Dalmatia and Galicia, a disease which results in a yellowing and blighting of the young plants. The organism that is responsible for this condition has been noted also on two other crop plants, tomatoes and cabbage. Preissecker (1905) noted that several weeds including goosefoot *(Chenopodium album)*, purslane *(Portulacca oleracea)*, and shepherd's purse *(Capsella bursa-pastoris)* are, in addition, subject to attack.

Symptoms.—This disease involves the root system and is confined to the smaller roots and root hairs of seedlings. Affected plants become pale green in color and fail to grow vigorously. Preissecker (1905) observed that it occurred especially on plants affected with root knot, and suggested the probable entrance of the organism through wounds made by the root knot nematode. Bensaude (1923), who observed the disease in greenhouses at the University of Wisconsin, states that the plants grow quite normally even though the roots proved, on microscopic examination, to be heavily infected. The lesions appear as slightly discolored areas. The epidermis, including the root hairs, and the outer layer of cortical cells are abundantly occupied by the fungus. It may penetrate to a depth of 4 or 5 cell-layers.

Cause.—This root disease of seedlings is caused by a simple phycomycetous fungus, *Olpidium brassicae* (Wor.) Dang. It was first described as *Chytridium brassicae* by the famous Russian mycologist, Woronin, who noted it on the rootlets of cabbage seedlings (Prings. Jahrb. Wiss. Bot. 11: 556. 1878). This name was changed by Dangeard to *Olpidium brassicae* (Wor.) Dang. (Ann. Sci. Nat. Ser. 7. 4: 327. 1886). Preissecker (1905) described as a new species *Olpidium nicotianae*, which appears to be only a variety or strain of *O. brassicae*.

The body of the fungus consists of a single globular cell that eventually transforms itself into a sporangium. Each sporangium usually measures 14 to 20 μ in diameter, but it may be considerably larger. Several sporangia

may occur within each cell of the diseased plant. In preparing to form spores, each sporangium develops an exit tube that may extend to the outside of the tobacco root, and then the uniciliate zoospores are formed. They are emitted in the soil and swim around in the films of water between the soil particles. Preissecker observed that the sporangia may open and emit zoospores within the parasitized cells, a fact which Bensaude confirmed.

Resting spores with a thick wall, covered with ridges, constitute the dormant stage of the fungus and may form within the cortical tissues. These resting spores serve to carry the fungus over periods unfavorable for the growth of plants or for its development within root tissues.

Presumably *Asterocystis radicis* de Wild., noted on tobacco seedlings by Peyronel (1920), is identical with *Olpidiaster radicis* (de Wild.) Pasch. and should be regarded as an *Olpidium*. It is not certain that it is specifically distinct from *Olpidium brassicae*.

Control.—Experiments designed to determine how to control this disease of seedlings have never been conducted. It is known that the fungus is soil-borne, and this fact indicates that control should be dependent on soil sterilization. It should not occur in beds that are properly drained or properly watered.

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Pythium Damping Off or Stem Burn

Several genera of soil-inhabiting fungi, including *Pythium*, are responsible for a diseased condition of tobacco seedlings that manifests itself by a decay of the stems at the surface of the soil. This type of disease is called "damping off" or "stem burn." Damping-off fungi belonging to the genus *Pythium* are of widespread occurrence and have the peculiar characteristic of being

able to attack a wide range of plants and, in addition, of being capable of living for indefinite periods on decaying vegetable matter. For these reasons they are essentially universally present in all arable soils.

Suscepts.—Plants aside from tobacco that are susceptible to attack by *Pythium* include the following: among field crops—corn, sugar cane, rice, cowpeas, soybeans, and flax; among truck crops—beets, cabbage, cauliflower, celery, cucumber, eggplant, pumpkin, squash, peas, strawberry, radish, turnips, tomato, and potato; among weeds—shepherd's purse *(Capsella bursa-pastoris)*, white mustard *(Brassica alba)*, and pig weed *(Amaranthus retroflexus)*; and among seedling trees—pine, spruce, Hevea rubber, and papaya. A list of suscept species in Sumatra given by Jochems (1927) includes *Pithecolobium saman, Albizzia falcata, Tectona grandis, Leucaena glauca, Crotolaria striata, Mimosa invisa, Imperata cylindrica,* and *Phytolacca decandra.*

Symptoms.—Damping off may appear at any stage of growth of seedlings in the bed. It may appear soon after germination or in rare cases may cause a decay of the stems of larger plants in the fields. It may involve patches of plants in seed beds, killing all seedlings as the patch enlarges. In Sumatra it oftentimes attacks the stems of plants the first days after transplanting, necessitating replanting, and resulting in irregularity in maturity of the crop (Jochems, 1927). The disease may spread rapidly in seed beds that are poorly ventilated, and too wet, due to poor drainage or excessive rainfall. Plants recently attacked are pale green and show a girdle of brown, decaying cortex extending both upward and downward from the ground level. Such plants wilt, eventually topple over, and the entire stem and leaves are decomposed.

The mycelium of the pathogen penetrates the cell walls, courses through the cells, and the invaded tissues become reduced to a formless mass.

Names of the Disease and Its Distribution.—The name "damping off" is commonly employed in English-

speaking countries for this seedling disease. Subramaniam (1919), in India, applied the name "*Pythium* disease," and Jochems (1927), in Sumatra, *stengelverbranding* (stem burn). Nolla (1932) states that the common name for the disease in Porto Rico is *salcocho* or *sancocho,* and that it is there a very destructive disease.

Although *Pythium* is ubiquitous in soils, the present range of this disease on tobacco includes Canada, Wisconsin, Connecticut, Porto Rico, Java, Sumatra, India, Philippine Islands, Nyasaland, Gold Coast, Rhodesia, Rumania, Germany, Russia, and Turkey.

Cause.—Several species of *Pythium* have been shown to be associated with damping off of tobacco. These include *Pythium deBaryanum* Hesse, *P. aphanidermatum* (Eds.) Fitz., *P. butleri* Subr., *P. myriotylum* Drechs., *P. Deliense* Meurs, and *P. perniciosum* Serb. Other names have been given to species of *Pythium* occurring on tobacco. Drechsler (1930) regards *P. myriotylum* as previously undescribed, although it was given the name *P. polyandrum*. Monographic studies of this genus by But-

Fig. 100. Damping off or stem burn of Deli tobacco seedlings caused by Pythium. (After Jochems.)

ler (1907) and by Matthews (1931) contain detailed descriptions of these species. The first of these, *P. deBaryanum*, described by Hesse (1874), is probably the best known. It can be recognized by its coarse, non-septate, granular mycelium, which may be seen when infected tissues are teased apart and examined under a microscope. Sporangia and oospores have been developed in culture and on decaying tissues. Meurs (1934) states that *P. Deliense* was formerly included with *P. deBaryanum*. Subramaniam (1919) found that fruiting structures of *P. butleri* are produced when affected parts are floated on water. This species appears to be identical with *P. aphanidermatum*. Serbinow (1912) decided that the fungus that is so widespread and destructive to seedlings of *Nicotiana rustica* in Crimea is specifically distinct from *P. deBaryanum*, and consequently regarded it as a new species, which he named *P. perniciosum*.

Contributory Factors.—All observations are in accord in showing that the moisture content of the soil and the humidity of the air are prime factors in influencing both infection and the course of the disease (Nolla, 1932). The widespread occurrence of species of *Pythium* in cultivated soils throughout the world shows that soil type is not a factor of importance. If soils of any type are wet for extended periods, and therefore poorly aerated, plants grown in them are liable to infection. This is especially true where high temperatures prevail. The pathogen is aerobic, and when the soil is of a texture to permit the water to pass through rapidly, the plants are less liable to become infected.

Since certain species of plants are especially subject to damping off, advantage may be taken of this fact in guarding against the disease. Jochems (1927) pointed out that *Leucaena glauca* and *Phytolacca decandra* are very susceptible. These two species are planted in Sumatra as borders to protect secondary vegetation on fallow tobacco lands against fires. When such borders are again planted to tobacco or are used as beds, severe losses from damping off may be expected.

Control.—The nature of the disease is such that attention must be centered on prevention in the seed beds. This necessitates, first of all, care in the selection of sites for beds so as to avoid excessively wet soils. Lands occupied in the previous year by crops especially subject to attack should be avoided. Jochems, in Sumatra, cautions against the use of fire borders occupied by *Leucaena glauca* and pokeweed.

No preventive measure can be substituted for soil sterilization. This can be accomplished by steam, where permanent seed beds are maintained, by heat, or by the use of formaldehyde. The methods of soil sterilization are discussed in a preceding chapter.

The avoidance of too thick sowing may aid in the circulation of the air around the seedlings and the maintenance of proper humidity conditions within the beds. Nolla (1932) emphasized that beds should be thinly sown.

If by removing the covers, withholding water, or by screening against rains, the plants can be hardened, they are less liable to damping off.

Jochems (1927 b) noted that the losses following transplanting of Deli tobacco can be largely prevented by shallow planting and by leaving the holes around the transplants open for eight to ten days. In field tests the losses of seedlings, in three fields planted in the usual way, were 23.1, 37, and 44.2 per cent, respectively, in comparison with 0.4, 0.6, and 5.1 per cent in similar plots planted the other way.

Serbinow (1912) recommends dusting the seedlings heavily with sulphur as a protective measure. Nolla (1932) recommends the employment of two sprayings with Bordeaux mixture at the rate of one-half gallon per square foot, one before sowing the seed and the other a week after their germination. Anderson (1934) secured excellent control of damping off in Connecticut by using one and one-half ounces of formaldehyde dust per square foot of surface, just before seeding.

There is little evidence of varietal resistance. Serbi-

now reported that the variety *Dubeck* is more severely injured than *Machorka*.

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RUST

A true rust, caused by one of the rust fungi, has been reported on tobacco from Italy and Brazil. The rust was designated *Uredo nicotianae* Anast., Sacc., et Splen. from collections made on *Nicotiana quadrivalvis* and *N. sylvestris*. Apparently only the red rust stage is known. It forms brownish pustules that are for the most part on the upper leaf surface on *N. sylvestris*, and on the lower leaf surface on *N. quadrivalvis*. In Brazil, near Piracicaba (Averna-Saccá, 1922), it occurs on cultivated Kentucky tobacco, but is not known to be of any economic importance anywhere. It produces brown sori on the lower leaf surface of cultivated tobacco, and the echinulate uredospores measure 24 by 32 μ.

This rust has been reported by Arthur (Arthur, J. C., *Manual of the Rusts in United States and Canada*, 438 pp., 1934) to occur in Southern California on *Nicotiana bigelovii*.

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Sooty Mold or Fumaggine

The sooty molds constitute a group of fungi that are especially abundant in tropical and subtropical climates. Many of them commonly occur on the leaves, twigs, and fruits of broad-leafed evergreens. As the name indicates, they produce a black film or crust that has the appearance of a layer of soot. This sooty layer consists of a weft of fungous threads that are superficial and that attach themselves by means of finger-like or expanded processes.

Sooty molds are commonly associated with plant lice (aphids) and scale insects, and subsist on the honey-dew secreted by these insects. The injury which they occasion is, therefore, caused not by parasitism but by shading.

A disorder of tobacco in Italy caused by one of the sooty mold fungi, *Apiosporium salicinum* (Pers.) Kze., was investigated by Inglese (1911). He identified the organism as *Fumago vagans* Pers. and noted that it was associated with *Aphis scabiosae*. No further study appears to have been made in Italy or elsewhere, although it has been reported from several countries. It has been encountered only once in the writer's experience, and is of such rare occurrence as to be regarded as of no economic importance

Inglese found that the aphids yield to spraying with 2 to 3 per cent tobacco decoction. This material does not leave any stain and consequently does not decrease the quality of the cured leaf.

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ANTHRACNOSE

Anthracnoses are of common occurrence on crop plants and are of the type of disease that occasions serious losses. In the case of anthracnose of tobacco, however, little damage is caused. No studies have been made of it except in Germany, where it manifests itself as a "stem and leaf scorch of seedlings." Nakata informed the writer that anthracnose of tobacco may cause serious damage in Korea (Chosen).

The causal fungus was first described by Averna-Saccá (1922) as *Colletotrichum nicotianae*, which is presumably identical with *C. tabacum*. Like many anthracnose fungi, the tobacco anthracnose fungus is seed-borne (Böning, 1932). He found that the incidence of primary infection to seedlings arising from seed saved from affected capsules is about 2 per cent. Notwithstanding this small proportion, the crowding of the seedlings in the beds makes possible the rapid spread of the disease, provided weather conditions are favorable.

Böning (1932) found that seed treatment was effective in control, but that complete control by seed disinfection was not possible. This is due to the fact that the fungus exists not only at the surface of the seed but also internal to it. The greatest degree of control was afforded by immersion of the seed for thirty minutes in either silver nitrate solution or in Uspulun-universal at a concentration of 0.25 per cent.

Copper salts employed as sprays and dusts reduced the amount of anthracnose on seedlings, in his experiments, and were beneficial when applied to the growing crop after it had been transplanted in the field.

Böning (1933) later reported another anthracnose on *Nicotiana rustica*, to which he gave the name *Gloeosporium nicotianae*. The conidia of this species are smaller

than those of *Colletotrichum nicotianae*, and the fungus forms light-brown, flocculent, slow-growing mycelium in culture in contrast with the greenish black, pulverulent, rapidly developing mycelium of *C. nicotianae*. Whether *Gloeosporium nicotianae* is pathogenic remains to be established. Since *Gloeosporium* and *Colletotrichum* are form genera in which morphologic and physiologic characters are known to vary widely, the identity of these forms on tobacco can be known definitely only after comparative studies of them.

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FIG. 101. Anthracnose on leaf of seedling from Japan. (From Nakata.)

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Alternaria Leaf Spot

Several apparently distinct species of Alternaria are associated with leaf spot diseases of tobacco. Whether the diseases are distinct because of the different environmental conditions in different countries, and whether the so-called species of Alternaria are really distinct, are problems that can only be solved by further investigation. Hopkins (1931) noted a disease in Rhodesia which he characterized by the presence of circular, light brown, zonate spots, about one-fourth inch in diameter. These spots gradually became pale with age. He attributed the occurrence of dark brown spots on the stems, mid-ribs, and seed pods of plants showing Alternaria spots on the foliage to the same cause. The disease appears first upon the lower leaves about the time that flowers are normally being formed, and, if conditions are favorable, it rapidly advances to the uppermost leaves and seed pods. Gulyás (1930), in Hungary, in accord with this observation, noted that the disease first appeared on weakened leaves, especially of the varieties Tiszai, Kapa, and Debrezin. Evidently this disease is identical with one which Preissecker (1903) had observed, in 1902, in Galicia and Dalmatia.

The cause of *Alternaria* leaf spot is *Alternaria tabacina* (E. and E.) Hori. Ellis and Everhart (1892) described a white speck disease of tobacco from specimens collected in North Carolina, whose causal organism possessed conidia 15 to 25 by 10 to 12 μ and other larger conidia 35 to 45 by 8 to 12 μ with a stalk 8 to 12 μ long. This is not a stalk but a tapering apex, since the conidia are attached at the blunt end. Two years later they (Ellis and Everhart, 1894) again described the same fungus, but used the name *Macrosporium tabacinum*, and gave the conidial measurements as 50 to 90 by 10 to 15 μ.

Hopkins (1931) found that the fungus varies greatly in size of conidia, thus allowing for the discrepancies in the conidial measurements. Hori (Formosa Agr. Exp. Sta. Bul. 1: pt. 1, 33 pp. 1908) noted that the conidia are borne in chains, and therefore he transferred the causal organism to *Alternaria* with the binomial *Alternaria tabacina* (E. et E.) Hori. Preissecker had also recognized the fact that the conidia are formed in chains, but he assigned it to *Alternaria brassicae* with a new varietal name *tabaci*. Gulyás was also aware of the fact that the fungus should not be assigned to *Macrosporium*, but being unaware that it had already been referred to *Alternaria* by Hori, he renamed it *Alternaria tabacinum* (E. et E.) Gulyás.

It is entirely possible that the perfect stage of this fungus is *Pleospora nicotianae* Averna-Saccá (1922), which was collected in Brazil on old lesions formed by *Alternaria tabacina*.

Control measures are entirely unknown.

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Pleosporose

Prior to the investigations of Ghimpu (1931) in Bulgaria, fragmentary notes had appeared from time to time calling attention to the occurrence of a disease on tobacco seedlings whose cause was identified as *Alternaria tenuis*

Nees. Splendore (1900, 1907) and Preissecker (1915) had noted that it produced brown lesions on seedlings in Italy, and in Dalmatia and Galicia, respectively. Ghimpu observed the disease also on essentially mature plants in the field and on the crop after it had been hung in the curing barns. The leaf lesions are brownish and become reddish with age. If high temperature and high humidity prevail, the conidia appear at the surface of the lesions as an olivaceous, velvety layer. This velvety layer consists of conidiophores that bear two types of conidia, small unicellular ones, and large pluricellular ones. The large ones are 25-68 by 10-15 μ, brown, muriform, and catenate. Ghimpu found that with the disintegration of the leaf tissues, perithecia were formed. Within these perithecia, asci 34-70 μ long, containing oval, muriform ascospores, were formed. This stage he identified as *Pleospora alternariae* Gib. et Giff.

The organism is not vigorously parasitic, as evidenced by Ghimpu's failure to secure infection on uninjured leaves. When the tissues were injured at time of inoculation on mature leaves, lesions developed. He also established that the disease is seed-borne, and emphasized the necessity of seed disinfection as an essential control measure. He employed for this purpose in a series of experiments, copper carbonate, copper sulphate, bichloride of mercury, formaldehyde, germisan, silver nitrate, potassium permanganate, and ceresan. The last two of these compounds were most effective, and he recommended their use in seed disinfection.

As a supplement to seed treatment, the use of trash from a diseased crop as a means of enrichment of the seed bed is to be avoided. Care as to the moisture and humidity conditions in the seed bed is suggested in order to render the seedlings less tender and thus less susceptible to this disease.

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PHYLLOSTICTA LEAF SPOT

A leaf spot disease of tobacco seedlings, of minor importance in the flue-cured-tobacco districts, is caused by *Phyllosticta nicotianae* E. and E. It occurs every season in beds sown with untreated seed. The disease is transported to the field on affected seedlings, and in several seasons has been observed to be present abundantly on the lowermost leaves of plants in the field. No intensive study of this disease has been made, doubtless because it is not known to cause serious damage. It has been re-

FIG. 102. Large brown lesions on leaves of seedlings produced by *Phyllosticta Nicotianae*. The centers become pale brown after the pycnidia have formed.

FIG. 103. Lesions of the Phyllosticta leaf spot disease on a leaf taken from the field in mid-summer.

ported from all of the states where flue-cured tobacco is grown and from Porto Rico, Nyasaland, Japan, and the Caucasus area in Russia. The present brief account is drawn from those of Wolf (1932) and Tsukyoku (1930).

Symptoms.—This leaf spot disease manifests itself by the presence of brown, irregular, zonate spots, 1 to 10 mm. in diameter. The lightest shades of brown are at the center, and the darker zones are toward the margins of the spots. The tissues surrounding these necrotic areas are yellowish green. Pycnidia, 15-150 μ in diameter and of approximately the same color as the tissues within which they are embedded, are sparingly present. These pycnidia can readily be seen with the aid of a hand lens.

Cause.—The organism responsible for this leaf spot is *Phyllosticta nicotianae* E. and E., described (Ellis and Everhart, 1893) from specimens collected in North Carolina. Wolf (1922) was unable to identify positively the organism which he studied because of inability to compare it with the type specimen, that appears to have been lost. The conidia of the fungus that he regarded as *P. nicotianae* measure 6 to 10 by 3 to 3.5 μ, about twice the dimensions recorded by Ellis and Everhart. Occasional conidia are one-septate, two-celled conidia being characteristic of *Ascochyta*.

Phyllosticta nicotianae should be examined in comparison with *P. tabaci* Pass., *P. capsulicola* Sacc. et Speg., and *P. nicotianicola* Speg. It is unlikely that all of these are distinct. It appears to differ from *P. tabaci* which causes the formation of irregular, dry, white areas in which black pycnidia are formed. The pycnidia of *P. capsulicola* form in groups and are beaked, and the conidia are curved and guttulate. *P. nicotianicola*, collected on *Nicotiana acutiflora* in Argentina, is morphologically most like *P. nicotianae*, but the lesions that it forms are different.

Control.—Observations indicate that *Phyllosticta* leaf spot is seed-borne, since it rarely occurs in seed beds sown with seed that have been disinfected prior to sow-

ing. Until more is known regarding the life history of the pathogen, definite recommendations for control, besides seed treatment, are without foundation.

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METALLIC MOLD

Attention was directed by Gandrup (1932) to a mustiness of tobacco in the Dutch East Indies, the cause of which was subsequently identified as *Blakeslea trispora* Thaxter. Jochems (1927), in Sumatra, found that it occurs on over-mature leaves in the field and in the drying sheds on leaves that have been recently harvested. Infection in the field follows weakening of the leaves, especially when the leaves have lost their turgor as the result of an infestation by the green tobacco bug, *Nezara viridis*. He noted that, when heavy infestations of this bug occurred, the entire tops of many of the plants came to be covered over thickly with the fructifications of the mold. It has a peculiar metallic lustre which may be employed as a means for its identification.

Several days after flower clusters have been broken off and have been thrown upon the ground, they are covered with a profuse coating of the fungus, which is essentially noticeable in early morning.

Jochem's attempts to inoculate healthy leaves led him to the conclusion that *Blakeslea trispora* is only weakly parasitic.

Blakeslea trispora was first described by Thaxter (1914) from cultures isolated from cowpea flowers. It is peculiar in that it possesses two types of sporangia, large ones with a columella, borne singly, and small ones without a columella, borne in dense heads. Weber and Wolf (1927) noted that the organism also forms zygospores and that it is heterothallic. This fungus occurs on fading

plants of a variety of species, and is tropical or subtropical in range. The writer has noted it as far north as Durham, N. C.

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BLOTCH OR SCAB

This disease is characterized by the presence of irregular, olivaceous blotches on the upper leaf surface of seedlings, and of similar blotches and streaks on the petioles and stems. During periods of excessive rainfall and high humidity, the petioles and stems may "damp off," and soft-rotted areas may appear on the leaves. When suitable aeration is provided or when slightly affected plants are set in the field, they may recover. Most injury develops in shaded beds or portions of beds that are shaded most of the time.

Tisdale (1929) isolated the causal fungus and proved its pathogenicity by artificial inoculation with pure cultures. He is of the opinion that the disease is similar to the one previously reported from Ohio, Kentucky, Wisconsin, and Connecticut, and attributed to *Fusarium affine* Sherb. or *Septomyxa affinis* (Sherb.) Wr.

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MISCELLANEOUS FUNGI PARASITIC ON TOBACCO

A considerable number of fungi, in addition to those that have been described in the foregoing accounts, are known to be parasitic on tobacco. None are reported to be important plant pathogens in areas from which they have been collected, but the probability exists that they

FIG. 104. Blotch or scab on a seedling taken from the seed bed. This condition is caused by *Septomyxa affinis*. (From Tisdale.)

FIG. 105. Sooty mold of tobacco.

might become destructive if introduced into other tobacco-growing areas or into other environments more suitable for their development. The following list includes those that have been briefly described:

1. *Ascochyta nicotianae* Pass. was first described as occurring on languid leaves of tobacco in Italy. It forms large, dry, irregular lesions in which the pycnidia are imbedded. The borders of these lesions are distinctly raised. This fungus has also been reported to be present in France, Austria, Germany, Russia, and Porto Rico.

2. *Botryosporium pulchrum* Cda. is semi-parasitic and occurs on tobacco that is being air-cured.

3. *Cercospora solanicola* Atk., first described as the cause of a leaf spot of potato in Alabama, has been collected on tobacco in Brazil.

4. *Cytospora nicotianae* Averna-Saccá occurs on the stems of tobacco and is known only from Brazil.

5. *Epicoccum purpurascens* Ehr. forms purple spots on the foliage and is known on tobacco from Italy alone.

6. *Macrophoma tabaci* Averna-Saccá has been reported on tobacco from Brazil.

7. *Macrophomina phaseoli* (Maub.) Ashby (*Rhizoctonia bataticola* Taub.) occurs on the roots of mature plants or on the moribund roots, not only of tobacco, but also of a variety of plants. These plants include cotton, soybean, cowpea, pepper, potato, bean, and tomato. It is suspected of damaging growing tobacco in Ceylon when peppers and tobacco are interplanted. It has been reported to cause injury to tobacco in Kentucky. Valleau collected affected plants in Kentucky, which have been examined by the writer.

8. *Moniliopsis aderholdi* Ruhl. occurs on tobacco seedlings grown under glass in Russia, causing their death, and is very destructive to seedlings of a variety of plants in Germany.

9. *Phyllosticta nicotianicola* Speg. causes the formation of dry more or less grayish spots, 1 to 10 mm. in diameter, on *Nicotiana acutiflora* in Argentina. These

spots become whitish when the pycnidia have appeared at the lower leaf surface.

10. *Phyllosticta tabaci* Pass. was reported from Italy on cultivated tobacco. Black pycnidia form in the irregular, dry, grayish lesions. This fungus is known to occur in Germany, Bohemia, Serbia, Hungary, Ukraine, and South Africa.

11. *Phyllosticta capsulicola* Sacc. appears on the capsules of cultivated tobacco. It was first described from Italy and has subsequently been collected in Hungary. The pycnidia are aggregated within the indefinite brown spots.

12. *Phyllosticta hainaensis* Frag. et Cif. is known only from the Dominican Republic, where it is associated, on dry leaves, with the ascomycete, *Sphaerulina hainaensis* Frag. et Cif., that appears to be the perfect stage.

13. *Septoria nicotianae* Pat. appears in concentrically zonate, pale brown spots on the leaves of tobacco in France.

14. *Septoria diversa* Sacc. et Syd. has been recorded as being pathogenic to *Nicotiana longiflora* in Argentina. It causes the formation of sordid red, grayish-centered spots with a narrow, reddish border, 1 to 20 mm. in diameter that are circular in outline. The black pycnidia are innate-erumpent with broad protruding pores.

15. *Stemphylium tabaci* Oud. has been reported in Holland as the cause of a leaf spot disease.

16. *Verticillium albo-atrum* Reinke et Berth. was isolated from a wilted tobacco plant in Tennessee. It appears doubtful that Averna-Saccá in Brazil observed the same disease.

IX

Diseases Caused by Nematodes

General Considerations.—The nematodes or eel worms comprise a group of animals, most species of which live in moist soil, in water, or in decaying organic matter. Some species, however, are important parasites, occurring either within the body or upon the body surface of higher animals or of plants.

Those which occur on plants effect several types of disease symptoms, dependent upon the portion of the plant which is the locus of attack. In the case of the "crimp" or "crinkle" disease of strawberries, the young leaves are primarily involved. In the case of the eel-worm disease of wheat, the inflorescence is involved, and the kernels or grains are transformed into hard galls which contain myriads of nematodes. In the case of the common root knot disease, irregular enlargements of the roots result from the attacks of the pathogen.

Apparently at least three species of nematodes produce injury to tobacco. The best-known and most widely distributed one causes the root knot disease, to be considered in the paragraphs that follow. Another species identified as *Tylenchus devastatrix* attacks the stems. Recently a third species, *Aphlenchus parietinus,* was reported to cause lesions, in Sicily, on the roots of a variety of tobacco called "*Zimma*" (Monastero, 1933).

Root Knot of Tobacco

The root knot disease of tobacco occurs throughout all portions of the world in which tobacco is grown that have a climate of similar mean temperature to that of the southern United States. The disease is characterized by knot-like enlargements on the root system, entirely unlike

those of any other disease which involves tobacco, with the exception of club root.

Suscepts.—The root knot organism, a nematode, is well-nigh omnivorous in its food habits, and many totally unrelated cultivated plant species are subject to attack, as are also many wild species including most of the common weeds. Our knowledge of the disease as it occurs on tobacco is due largely to the work of Schoevers (1917) and van Breda de Haan (1900). The following species are selected from among a known list of over 500 suscept species: (1) field crops, including alfalfa, clover, cotton, cowpeas (except the varieties Iron, Brabham, and Monetta), sugar beets, soybeans (except Laredo), tobacco, and vetch; (2) truck crops, including asparagus, beans, beet, cantaloupe, carrots, celery, cucumber, eggplant, lettuce, okra, onion, peas, pepper, potatoes, salsify, spinach, strawberry, tomato, and watermelon; and (3) fruit trees, including peaches, pecans, fig, cherry, plum, and walnut. The cereal crops, including corn, wheat, rye, oats, barley, and sorghum; many grasses; and such leguminous crops as peanuts, velvet beans, beggar weed, Laredo soybeans and certain varieties of cowpeas are immune or sufficiently resistant that they may be grown in infested soils.

Symptoms.—Tobacco in any stage of its growth is subject to attack by the nematode, *Caconema radicicola* (Greef) Cobb, resulting in an involvement of the root system alone. In the case of certain other crops with underground stems, as the Irish potato, these organs also become affected. Another species of nematode, *Tylenchus devastatrix*, has been shown, in the East Indies, to attack the stems of tobacco (Schoevers, 1917). Injury from root knot, caused by the common nematode, is in many cases entirely overlooked because of the fact that no enlargements are formed on the parts above ground. Badly affected plants are stunted or dwarfed and wilt readily, especially in dry, hot weather. They may recover their turgidity at night, but after wilting for several days in succession the leaves become dry, and the plants may be killed.

The roots are irregularly enlarged and deformed. These enlargements may vary in size from scarcely noticeable swellings on the smaller roots to extensive elongated swellings an inch or more in diameter on larger roots. The extent of infestation of the soil together with the prevalence of conditions favorable to the organism govern the number and size of the galls, i. e., the extent of involvement of the roots. In extreme cases nearly the entire root system may be involved.

Microscopic examination of affected tissues shows that they are hypertrophied as a result of the irritation incited by the presence of the pathogen. The nematodes feed by means of a buccal spear by which they pierce the cells and suck out the contents. The parenchyma cells become abnormally large, and each possesses several nuclei instead of a single one. Sometimes only a few adjacent cells are involved, sometimes a large number. In the latter case the enlargement of the cortical cells leads to a displacement and distortion of the elements of the vas-

FIG. 106. Root knot of tobacco. The enlargements on the roots are due to nematodes or eel worms.

cular bundles. In consequence there is a marked interference, both with the transfer of water and of food by the vascular system and with the development of root hairs needed for the absorption of water and mineral salts from the soil.

The amount of enlargement and distortion depends in part upon the number of nematodes attacking the root at any one point. As the cells swell, they enclose the nematode to varying degrees and may finally completely invest it.

The Disease.—Among the names applied to the root knot disease are "root gall," "big-root," "club-root," and "eel-worm disease." It appears to have first been noted on tobacco by Janse in 1892 in the East Indies. It was earlier known, however, on other crops. The famous English mycologist, M. J. Berkeley, in 1853, first described root knot on greenhouse plants in England, and Greef on several out-of-doors plants in Germany in 1864. Since then it has been observed on many kinds of plants, as previously stated, in all parts of the world. These places of collection include France, Italy, Austria, Holland, Sweden, Russia, Egypt, Algeria, Madagascar, South Africa, Java, Sumatra, China, Japan, Brazil, Argentina, Chili, Mexico, the West Indies, Australia, and the United States. Its original home was probably the Tropics, whence it has been introduced and disseminated into cultivated lands in all temperate portions of the world.

Under normal weather conditions root knot does not appear to cause appreciable damage to tobacco. When the roots are severely involved, however, and a period of dry weather prevails, the yield is reduced considerably. In seasons extremely deficient in rainfall, the crop has been observed in North Carolina to dry up prematurely, whereas near-by fields of tobacco whose roots were normal or only slightly affected withstood the drought.

Cause.—Root knot is caused by a nematode or eel worm, a minute animal called *Caconema radicola* (Greef) Cobb. According to Bessey (1911), this nematode was

first described in 1872 by Greef as *Anguillula radicicola*. In 1879 Cornu described this nema, which he observed on sanfoin, as *Anguillula marioni*. The first extensive study of the organism was made by Müller, who gave it the name *Heterodera radicicola* (Greef) Müller by which it is the best known at present. Cobb, in 1924, separated it from *Heterodera* and used it as the type of *Caconema*, with the new binominal *Caconema radicicola* (Greef) Cobb.[1]

Several other names have been employed at various times by different investigators. The organism which Treub described in 1885 on sugar cane as *Heterodera javanica* is probably identical with *C. radicicola*. Neal's *Anguillula arenaria* from Florida, described in 1889, is another synonym, as are Cobb's *Tylenchus radicicola*, in 1890, from New South Wales, and Göldi's *Meliodogyne exigua*, in 1892, on roots of coffee from Brazil.

Mature nematodes of the two sexes are morphologically distinct. The males are slender and measure 1200-1500 by 30-36 μ. The females are pear shaped and measure 400-1300 by 270-500 μ. A female may lay as many as 500 eggs, which may be expelled from the body of the gravid mother after they have segmented. The larvae emerge within the tissues of the root knot, but may escape in the soil and survive for several months. They are able by means of a buccal spear to pierce young root tissues and start anew the formation of root knots. A life cycle may be complete within 4 to 5 weeks, so that in warm climates 10 to 12 generations per year are possible.

Contributing Factors.—Plants grown on light sandy soils are more seriously affected with root knot than those grown on stiff, clayey soils. Soils which are marshy or wet, or else excessively dry are unfavorable for the pathogen. Plants grown in infertile soils or in fields which are impoverished are more seriously affected than those well supplied with mineral salts. While nematodes can survive in frozen soil, they become inactive when the

[1] According to the Plant Disease Reporter **17**: 64-96, June 15, 1933, *Heterodera marioni* (Cornu) Goodey is the proper designation for the root knot nematode.

soil temperature approaches 50° F. High soil temperatures for extended portions of the year, such as are attained in the southern United States, favor the development of the organism.

Control.—It is essential first of all in the control of root knot of tobacco that appropriate precautions be taken to prevent infection of the seedlings in the seed bed through use of infested soils. When seed beds are located on land that has not been cultivated and that is remote from a source of contamination, the seedlings will most probably be free from infection. When this procedure is not feasible, the soil should be sterilized before seeding by means of steam, heat, or chemicals. Jack (1927) noted that root knot in Australia is traceable to the use of infested seedlings, and that if healthy seedlings are set into average tobacco lands, damage is unlikely to occur.

Once a field has become seriously infested, the only satisfactory way of controlling root knot is by means of a system of rotation in which crops not susceptible to the root knot disease are grown for a period of years. Usually three years are sufficient, when clean cultivation is practiced, to keep down weeds which serve as food plants for nematodes. Cropping with corn, wheat, oats, rye, peanuts, velvet beans, and Laredo soybeans during this period is recommended.

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X

Diseases Caused by Parasitic Flowering or Seed Plants

Members of several families of seed-bearing plants have long been known to depend upon other seed plants for their food materials. Some are entirely lacking in chlorophyll and are thus true parasites. Such parasites rob the green plant to which they are attached of all elaborated food materials necessary for their growth. Others only rob the plant to which they are attached of water and minerals in solution. They possess chlorophyll and are therefore able to make their own food from these crude materials. Still others take both water and dissolved minerals and elaborated foods from their host species.

Tobacco seems to be subject to attack by members of two of these families of parasitic flowering plants, the *Cuscutaceae* or dodders, and the *Orobanchaceae* or broomrapes. The parasitic members in the former depend upon the tobacco plant, not only for water and dissolved minerals, but also for elaborated foods; the members of the latter rob tobacco plants of elaborated foods and are wholly unable to make their own food. Because of their peculiar food habits the members of these two families have been extensively studied. Most of the investigations, however, have been concerned with their morphology and physiology, not in conection with their occurrence upon tobacco, but upon other species of plants, mostly herbaceous ones. Presumably the anatomical and physiological relations between parasite and host among these parasitic flowering plants are quite the same whether one is dealing with their occurrence upon tobacco or upon such plants as clover, alfalfa, hemp, or goldenrod.

Dodders

General Considerations.—The dodders belong to the family *Cuscutaceae*, and were at one time treated as a tribe or sub-family of the morning-glory family, *Convolvulaceae*. Their separation as a distinct family is justifiable, since their peculiar structure and life history are an expression of their adaptation to food habits quite unlike those of the morning-glories. The dodders with which everyone is most familiar are those that occur on alfalfa and clovers, upon which crops they appear as conspicuous tangles of yellow vines and upon which they are a serious menace in many places.

Some species of dodders confine themselves to a single host species; others can attack a wide variety of plants in widely separated families, even both herbaceous and shrubby species, with no evidence of preference. Many different species of dodders have been described. Yuncker (1921) listed 54 species and 42 varieties of dodders as occurring within North America.

Appearance of Dodder.—As has been stated, dodder looks like a mass of intertwined yellow strings. For this reason it has been given a number of common names, including "gold thread," "hair weed," "love vine," "bind vine," and "strangle weed." The vines or stems are usually yellow, but they may vary to shades of orange, pink, or purple, or may in some cases be almost white. They appear to have no leaves, but close examination reveals the presence of tiny scale-like leaves. Beginning about mid-summer, clusters of tiny yellow, white, or pink flowers are developed, from which capsules bearing seed arise. Dodder on tobacco has been noted in most tobacco-growing countries. Preissecker (1904) has noted it on mature plants in Galicia and Dalmatia, and Atanasoff (1930), in Bulgaria. It is not common anywhere, however, on plants in the field because of tillage practices, but has been observed in seed beds of flue-cured-tobacco seedlings in several localities in North Carolina.

The Parasite and Its Life History.—Several species of dodder, including *Cuscuta epithymum* Murr., *C.*

monogyna Vahl., *C. europaea* L., and *C. arvensis* Beyrich., have been reported to parasitize tobacco. It is not known whether additional species are involved. *Cuscuta alba*, reported on tobacco, is synonymous with *C. epithymum*. Dodders are first evident in spring after other herbaceous vegetation is well started. The seeds germinate in response to suitable temperature and moisture conditions, just as is the case with those of other seed plants. The seedling is a thread-like structure which may rest upon the soil or be only slightly buried beneath the surface. The upper end comes to assume a vertical position; and in elongating, the tip spirals in search of a support. If it contacts a suitable host, haustoria or organs for absorption of food form on the side in contact with the host. If in spiralling only dead stems or some unsuitable host are reached, the seedling will die as soon as the supply of reserve food in the seed is exhausted. As soon as the haustoria penetrate a suitable host, the portions of the seedling below the haustorium and in contact with the soil shrivel and die. Then in the normal course of development the seedling grows into long threads that bear dense clusters of flowers and eventually produces seeds.

The Haustorium and Its Development.—Anatomical studies of the relation of dodder to tobacco have never been made. It attaches itself, though, to both stems and leaves, and presumably establishes connection with the vascular system of tobacco as has been described by Thoday (1911) for *Cuscuta* on other host plants, especially *Salvia*. She states that "a sucker-like organ first arises from the epidermis of the mother stem and adheres firmly to the host plant, its formation is then followed by the ingrowth of the true haustorium, which has originated endogenously, mainly from the cortical region just outside the pericycle, and seems very properly to represent an adventitious root. The cells of the sucker or 'prehaustorium' dissolve their way into the host plant, partly by pressure, partly by the excretion of a ferment, and into the place thus made the haustorium grows, en-

larging the opening and becoming surrounded by a mass of compacted dead cells."

Once penetration of the host plant has been accom-

FIG. 107. Dodder on seedlings that had been left in the seed bed after the season for transplanting. The knot-like structures are clusters of flowers of Cuscuta.

plished, a bundle of hyphae-like cells is produced by the superficial cells of the haustorium. These advance into the host tissue and may finally penetrate to the pith. Meanwhile some of the central cells of this bundle become applied to the water-conducting elements of the host, become differentiated into tracheal tubes, and thus form a continuous connection for the passage of water from the host to the parasite. Other elements of the bundle grow into intimate contact with the elaborated food-conducting elements of the host, become differentiated into sieve tubes, and thus form a continuous connection between the host and the dodder for the passage of elaborated food. When this has been accomplished, there is a physiological continuity between the dodder and the plant which it is attacking.

CONTROL.—The eradication of dodder depends upon the destruction of its host plant before seeds of dodder are formed. No difficulty need be encountered in preventing the occurrence of dodder in seed beds. This can be accomplished by utilizing sites for seed beds in which dodder did not grow on various weeds during the previous year or from which it was completely rogued. If dodder appears on the seedlings, all affected ones should be discarded.

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BROOM-RAPES OF TOBACCO

Members of a group of parasitic flowering plants, the *Orobanchaceae*, commonly called broom-rapes, are known to attack tobacco and other crops. Certain species of broom-rapes parasitize a rather wide range of plants, while others are restricted to a few or even a single susceptible species. These curious plants are unable to make

their own food, contrary to the condition prevailing among flowering plants, but attach themselves to the roots of some green flowering plant, and thus are nourished at the expense of the plant upon whose roots they are fastened.

Suscepts.—Several species of broom-rapes are known to attack tobacco, and none of them is confined exclusively to this suscept species. The one most commonly present on cultivated tobacco within the United States, *Orobanche ramosa,* parasitizes also *Nicotiana glauca* and *N. rustica.* It occurs, in addition, on hemp *(Cannabis sativa),* tomato *(Lycopersicum esculentum),* potato *(Solanum tuberosum),* parsnip *(Pastinacea sativa),* rape *(Brassica rapa),* and a considerable number of weeds and garden flowers. Constancis (1909) recorded its occurrence also on maize *(Zea mays)* and grape (*Vitis* sp.) in southern France.

Symptoms.—Plants parasitized by broom-rapes usually occur in small patches, and more rarely are distributed singly throughout the field. The presence of broom-rapes can be recognized by the occurrence of whitish to yellowish plants about 6 to 18 inches tall which arise from the soil at the base of tobacco plants. Their leaves are scale-like, and from mid-summer to the close of the growing season the stems are closely crowded with flowers. If the soil is removed from the base of the affected tobacco, the broom-rape will be found to be fastened to the roots of the tobacco, often as many as a dozen or more on a single tobacco plant. Since all of the food for the parasite is supplied by the tobacco, the crop is injured in proportion to the size and number of broom-rape plants which are being nourished at the expense of any individual tobacco plant, together with the general prevalence of the parasite. The plants become infected soon after being set in the fields, and in extreme cases may be injured to the extent of reducing the value of the crop one-half (Garman, 1903). In other cases, and at the opposite extreme, there may be little or no appreciable damage to the quality and quantity of the crop.

FIG. 108. Young broom-rape plants attached to the roots of a tobacco plant. (From Lehman.)

FIG. 109. A, entire broom-rape plant in flower, attached to the root of a tobacco plant. B, an enlarged stem showing the leaves and flowers of the broom-rape plant. (After Garman.)

NAMES AND HISTORY OF THE DISEASE.—This disorder is most commonly called "broom-rape" or "Orobanche disease," following either the popular name or the scientific name of the parasite. In India it has been designated *tokra*. It occurs in all of the different tobacco-growing sections of the United States, India, Europe, and Japan and Cuba, to a limited extent. It does not, however, appear to be wide-spread and seriously destructive in any country. It is not known to be of consequence anywhere within the United States except in the Burley tobacco-growing areas in Kentucky, where hemp is also grown. It was introduced here with the hemp seed and attacks tobacco only when grown on infested soil.

CAUSE.—The broom-rapes, which are widely scattered over the earth, comprise eleven genera and approximately two hundred species. Of these, six species are known to occur on tobacco. Species of the genus *Orobanche* have been assigned by various investigators also to *Phelypaea* and *Aphyllon*. Goutiere (1900) used the name *Phelypaea ramosa* C. A. Mey, for which the name *Orobanche ramosa* L. is preferable, for the broom-rape of tobacco in southern France, as was done also by Constancis in 1909. *Orobanche ramosa* is cosmopolitan in distribution. It has been reported on tobacco from middle and southern Europe, northern Africa, and India. *Orobanche ludoviciana*, properly *Aphyllon ludovicianum* A. Gray, has been observed to be most common in Kentucky. *Phelypaea indica* G. Don, better known as *Orobanche aegyptiaca* Pers., occurs in India; and *O. cumana* Wallr. occurs on *Nicotiana rustica* in southern Europe, northern Africa, Asia Minor, and India. *Orobanche muteli* Schultz occurs in southern Europe and Kapland.

As has previously been indicated, *Aphyllon ludovicianum* is a whitish, fleshy stemmed plant about a foot in height. In place of leaves it bears scale-like bracts which become brown at the tips as they mature. The flowers are about a half-inch long and are white or faintly purplish, arising singly in the axils of the scales. The seed

pods are ovoid, approximately a fifth of an inch long, and contain several hundred minute seeds.

The seeds seem never to germinate unless they chance to lie near or in contact with the roots of some plant from which nourishment could be secured. Strangely the roots of plants which they can not attack are without effect in stimulating germination. Garman (1903) has noted that unless the seeds come in contact with the root hairs of a suitable food plant they may remain dormant in the soil for a period of thirteen years, even though conditions of moisture and temperature favorable for germination prevail.

Upon germination the cylindrical radicle of the young broom-rape becomes attached to the root of the tobacco plant, whereupon a swelling arises on the tobacco root, surrounding the radicle like a shallow disc or cup. There is thus established an intimate contact of the broom-rape and the tissues of the tobacco root from which the broom-rape absorbs its food. The young bud then starts to develop, unfolding bract after bract, and eventually appears at the surface of the soil, having then somewhat the appearance of a small shoot of asparagus. Meanwhile the broom-rape has formed, in addition to the primary root, other blunt-pointed, simple roots which extend outward and come in contact with other tobacco roots, to which they become attached in the same way as did the primary root. Numerous swellings are thus formed as the broom-rape becomes attached to a considerable number of fibrous roots. About six weeks to two months after the broom-rape appears at the surface of the soil, the seed pods have been matured, and the seeds are being scattered on the soil where they remain dormant awaiting the setting of new crop of tobacco.

Control.—Manifestly the control of broom-rape centers on the prevention of the introduction of its seed along with those of some susceptible crop other than tobacco, since there is little likelihood of its introduction with tobacco seed. Garman has attempted, without success, the use of various means of removing broom-rape

seed from crop seed, such as by floatation in brine and by destruction with chemicals and hot water.

He has also attempted to destroy the seed in infested soils by applying salt, copper sulphate, iron sulphate, or quicklime, but these means appear to be impractical.

If one is dealing with fields which are already infested, the planting of such land for several years in succession with crops not susceptible to attack should be beneficial. Such plants as might appear parasitic on weeds should be prevented from setting seed.

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STRIGA DISEASE OR WITCHWEED DISEASE

The parasitic weed, *Striga orobanchoides*, is another member of the broom-rape family, *Orobanchaceae*, and is of particular interest because it parasitizes the roots of tobacco. It has been reported in South Africa to attack *Nicotiana rustica*, but is of rare occurrence there (Moore, 1926).

Species of the genus *Striga* are fairly common in tropical Africa, Egypt, Madagascar, Arabia, Ceylon, India, Siam, Java, and China, and have been especially studied in their relationship to sugar cane and other grasses (Bell and Cottrell-Dormer, 1922, and Pearson, 1929).

APPEARANCE.—The above-ground stems of *Striga* are green and are clothed with small elongate leaves, opposite below and alternate above. The underground portions of the stems are white and bear white scale-like leaves. The roots are light colored and contrast sharply with those of the tobacco plants. When the parasite is dried it becomes bluish black. Small sessile flowers, borne in interrupted spikes, appear in late summer. The

seed capsules produce extremely large numbers of tiny seed. Pearson (1929) says that these seed germinate in spring in the presence of a host plant, and that small spherical haustoria form where the root of the seedling comes in contact with the host root. This haustorium attaches itself to the root, forms slender outgrowths which penetrate the host root tissues, and becomes applied in such a manner as to effect an intimate contact with the vascular tissues and thus make a continuous system between host and parasite. The parasite forms root hairs which enable it to absorb water. Its cells contain chlorophyll, and it can thereby make a portion of its own food. The remainder is taken from its hosts.

Plants parasited by several Striga plants could be expected to be stunted. Affected plants occur singly or in small patches.

Control.—Striga is an annual, and its survival is dependent upon the production of seed. For this reason plants should be kept from setting seed. Infested soils, if planted with crops which are not attacked, as with legumes, should become freed from the parasite.

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XI

Decays of Tobacco during Curing, during Fermentation and Storage, and after Manufacture

Introduction.—After tobacco has been harvested it must be cured, either by exposure to natural environmental conditions as obtain when it is hung in sheds or in the open, or else by exposure to artificial environmental conditions as obtain in flue-curing. During curing of the former type, tobacco is prone to attack by many of the organisms that occurred as parasites upon it in the field, because many of the same species are capable of thriving on moribund tissues or on tissues that are dead. In addition, many species of molds that act only on dead tissues find lodgment upon the harvested leaves and produce decays that are injurious to the quality of the cured product. Injuries of this type are largely prevented when tobacco is flue-cured, because the temperatures maintained in curing completely sterilize the tissues. Flue-cured tobacco, however, becomes contaminated again while it is coming into "order" to make it possible to remove the tobacco from the curing barn without breaking the leaves. It is also exposed to contamination with a variety of molds and bacteria while it is being packed away in the storehouse, while it is being graded preparatory for market, and while it is being sold on the warehouse floor. These organisms may remain on the tobacco while it is afterward being prepared for storage and subsequent ageing. If environmental conditions in storage are favorable for the development of molds and bacteria, they may produce undesirable modifications of color, texture, and aroma.

Some types of air-cured tobacco, especially cigar to-

baccos, are fermented or sweated before being used in the manufactured product. Fermentation involves the activities both of enzymes within the tissues of the cured leaves and of microörganisms. These microörganisms have lodged on the tobacco leaves in handling, and are able to produce the desired chemical changes within the leaves only when suitable temperature and moisture conditions are provided. Proper fermentation has become an art. By means of it raw tobacco can be transformed into a ripened, mellow, aromatic product within a few weeks' time. The ageing of flue-cured tobacco, however, requires several years' time ordinarily, and it is not primarily dependent upon microbial activity. A comprehensive study of fermentation of tobacco is contained in a recent report by Smirnov (1933), to which the reader is referred.[1]

The damage to tobacco after harvest may, for convenience, be arbitrarily divided into three types: (1) damage during curing, (2) damage during fermentation and storage, and (3) damage to the manufactured tobacco. The following brief discussion deals with the information available on damage that occurs at these three stages.

Damage During Curing.—It has previously been pointed out that such organisms as the black shank, the frog-eye, the *Sclerotinia*, and the gray mold fungi may continue to develop after the crop has been harvested, so that these findings need not be repeated at this point. In addition, there are a number of disorders that are not so well known to investigators but with which growers are more or less familiar. These injuries will be briefly discussed under the caption of their common names: (1) shed burn or pole sweat, (2) stem rot or rib rot, (3) wet butts or fat stem, and (4) white veins.

Shed Burn or Pole Sweat

Appearance.—Johnson (1924) characterizes this type of injury to air-cured tobacco as productive of a darken-

[1] Smirnov, A. I., The physiologico-biochemical principles of tobacco curing and fermentation. 507 pp. illus. Krasnodar. 1933.

ing of the leaf or of large areas of the leaves, accompanied by a tendency of the involved portions to dry out too rapidly and to become brittle. When such leaves are disturbed in any way in handling, the affected parts break into pieces and fall out.

Cause.—It has been pointed out that shed burn is, in some cases, produced by *Botrytis cinerea* Pers. and *Sclerotinia libertiana* Fkl., but experimental evidence (Johnson, 1924) indicates that other fungi, notably species of *Alternaria*, *Fusarium*, and *Aspergillus*, may also be concerned. The organisms are only able to attack leaves that have become yellow or are past the yellow stage in curing. If at this time the relative humidity is above 95 per cent, and there is little circulation of air in the drying shed, moisture can collect on the closely hanging leaves, and conditions are ideal for the development of shed burn.

Control.—Control of shed burn depends upon adequate circulation of air throughout the drying shed. It may be necessary during rainy, foggy weather to raise the temperature within the sheds by artificial heat. If the temperature is raised, it increases the ability of the air to hold moisture and at the same time sets into motion currents of air that tend to carry away the excess moisture. Several small open fires at different points in the shed, with metal sheets above the blaze to deflect it, should adequately warm the air. Sometimes charcoal braziers are used for this purpose. The ventilators, except those in the roof, should be closed at such times.

Stem Rot or Rib Rot

Appearance.—The same conditions that favor the proper curing of the blade or laminar tissues of the leaf also favor the curing of the midribs. Since, however, the ribs are thicker, they dry out more slowly. It may happen, therefore, after curing has progressed to the point at which there is no longer danger of shed burn injury to the blades, that the midribs will become involved in de-

cay. Decay of the midribs at this stage of curing is called stem rot.

Cause.—It is not known definitely what organisms are responsible for stem rot of curing tobacco. In addition to those that occur on the blades in shed burn, there may be species of *Cephalothecium* and *Trichothecium* on decaying stems. The same environmental conditions that facilitate the development of shed burn also favor this stem rot.

Control.—It is necessary in order to control this form of decay to hasten drying. This may be done by using the same methods as are employed in avoiding or checking shed burn.

Wet Butts or Fat Stem

Appearance.—Whenever the basal part of the midribs and larger veins of cured leaves are wet, soggy, and discolored, the disorder called wet butts is indicated. In some cases entire leaves are involved, and they are then entirely worthless. If only the base of the leaf is involved, the leaf may be dried out during stripping and grading with little loss.

Cause.—Observations indicate that wet butts is most prevalent in crops that are subjected to freezing weather before curing is completed. It is not known definitely, however, that the disorder is caused by the freezing of the partly cured crop.

Control.—Avoidance of wet butts is possible by harvesting the crop sufficiently early to permit it to be cured before the advent of freezing weather, or by hastening curing with artificial heat.

White Veins

When tobacco cures too rapidly as during dry seasons, the outer tissues of the veins become whitened, hence the name "white veins." This whitening appears when a layer of air forms, as the result of a differential rate of drying, between the epidermal portions and those subjacent to the epidermis. Growers have learned to

avoid this condition by keeping the ventilators closed during the daytime, and by sprinkling the floors of the curing barns to raise the relative humidity. Then at night the proper ventilation is provided to maintain a uniformly gradual drying.

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DAMAGE DURING FERMENTATION AND STORAGE.—Considerable investigation has been made of mold spoilage of air-cured tobacco during fermentation and storage. The most important of these disorders are black rot, stack mold, and mustiness or white mold. Each of these will be considered in turn before discussing spoilage of flue-cured tobacco, about which there is little published information. Besides these decays of air-cured tobacco, there are others less well known but with which such cosmopolitan organisms as the following are associated: *Rhizopus nigricans* Ehrb., *Mucor mucedo* L., *M. racemosus* Fr., *Aspergillus glaucus* (L.) Link, *Penicillium glaucum* Link, *Cladosporium herbarum* (Pers.) Link, *Cephalothecium roseum* Cda., *Alternaria tenuis* Nees., *Catenularia fuliginea* Saito, and *Fusarium roseum* Link.

Recently van Beyma Thoe Kingma (1933 a) isolated several species of Monilia from tobacco in Holland. Three of these, *Monilia cerebriforme*, *M. macrospora*, and *M. microspora*, he described as new species. Another, *Oospora medoacensis* Sacc., he transferred to *Monilia* and called it *Monilia medoacensis* (Sacc.) van Beyma Thoe Kingma. He (1933 b) also described *Cephalosporium tabacinum* which he isolated from the tissues of a diseased tobacco plant.

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Black Rot

Cigar tobacco, during fermentation or sweating, is subject to a decay known as black rot. Proper fermentation is conditioned by suitable moisture and temperature relations, and black rot appears when these relations are not properly maintained. An understanding of this matter can best be had when once the practices in handling cigar tobacco are recalled. After the crop has been air-cured from exposure for about two months in the barns, the leaves may be so dry and brittle that they would crumble to pieces if handled. For this reason they must be moistened to permit them to come into "order" or "case." The leaves are then pliable and may be stripped from the stalks and packed in bundles. After this, it is graded and tied into "hands," whereupon it is packed under pressure into boxes or cases with the butts toward the outside and the tips toward the center of the box. Each case contains from 300 to 400 pounds. At this stage the tobacco is ready to be sweated, and is stored where temperatures can be regulated to permit a forced sweat, or it may be placed in storage to undergo a natural sweat.

In some places, instead of packing the tobacco into cases, it is piled into stacks of 2,000 pounds or more where it undergoes a bulk sweating. When the temperature within the stack has reached 100° to 120° F., the piles may be taken down and repiled in a similar manner. It may be necessary to repeat this several times before fermentation has been completed and in order to prevent black rot.

Appearance.—Experienced packers are able to recognize the presence of black rot within the bulk or case by a characteristic odor. Decayed leaves have blackened areas, especially at the tip ends of the leaves. These blackened areas are dry and will break up into small fragments when the hands are disturbed. Usually this decay develops in small areas throughout the bulk or case, but in some instances the entire central portions of the case are decayed, and there remains a shell of undecayed leaves toward the exterior of the case. The decayed areas

FIG. 110. Two hands of air-cured tobacco that became affected with black rot during fermentation. The blackened portion of the tips of the hand at the left is decayed. The hand at the right is similar to the other and shows that the tips fall away when the hand of tobacco is shaken or disturbed in packing. (From James Johnson.)

within the case may thus appear as pockets of black rot, and the hands are in consequence frayed and tattered when they are removed from the case or are repacked.

Cause.—Black rot is caused by a cosmopolitan mold, *Sterigmatocystis nigra* v. Tiegh. This mold is of common occurrence wherever vegetable matter is being decomposed at high temperatures, and is a common contaminant of laboratory cultures. Such fruits as figs and pomegranates, as they approach maturity, are rotted by it. It also serves a useful function in the manufacture of gallic acid from tannin, in the saccharification of starch, and in the manufacture of oxalic acid. It has the ability to thrive and to form an abundance of black conidia on a wide variety of substrata.

Conditions Favorable for Black Rot.—Johnson (1914) found that *Sterigmatocystis nigra* thrives at a wide range of temperatures, so that it is impossible, in the fermentation of tobacco, to avoid temperatures favorable for the mold. Advantage may be taken, however, of the length of time that the tobacco remains at this favorable temperature. In natural sweating, a period of from one to two months is required to bring the temperature from 86° to 104° F., depending upon the out-of-doors temperatures. In a forced sweat, depending upon how much heat is applied, a similar temperature may be reached in from one to two weeks. In bulk fermentation a temperature of 104° to 140° F. may be reached in from three to six days. In this last method of sweating the bulk rapidly passes through the temperatures favorable for the mold, and the tobacco may be repacked before black rot starts. Moisture will be lost during repacking, and the possibility of decay in the next bulk will be much decreased.

Control.—The simplest method of control would appear to be the destruction of the mold, and thus the prevention of black rot. This is impossible since the mold is brought into the warehouse along with the tobacco upon which it is always present. A thorough cleaning of the warehouse and the removal of all refuse from previous crops are, of course, advisable in order to decrease the

amount of inoculum. The most essential feature is to give attention to the moisture content of the leaves when they are packed into cases, and the temperatures that obtain during fermentation. Bundles in too high case should be left to dry out before being put into boxes. The moisture content may be reduced by one bulking before the hands are placed in boxes. If the packs have a moisture content that is excessive, bulk fermentation instead of case fermentation, with several bulkings and rehandlings, may be employed.

Storage of tobacco in boxes in rooms sufficiently cool to check the growth of the black rot fungus, and to permit the tobacco to dry out slowly is attended with the danger of favoring the development of "mustiness," or "white mold," another storage disorder.

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STACK MOLD

Among the molds that occur in air-cured tobacco which is stacked to promote fermentation or is baled for shipment is one to which attention was first directed, in Italy, by Splendore (1899). He called it *fioritura* or *fiorume* because of the grayish to white moldy spots that formed on the leaves. The fungus that causes this condition was described subsequently by Pezzolato and Saccardo as *Oospora nicotianae*.

What appears to be the same mold was noted, in Sumatra, in 1921-1922, by Palm and Jochems (1923) on Deli tobacco. They, however, regarded the organism with which they were working as an undescribed form and gave it the name *Andreaea deliensis*. Because the sclerotia and conidia which it formed were somewhat like those of *Penicillium*, they placed it near this genus among

the *Aspergillaceae*. Soon afterward they proposed that the name *Andreaea* be changed to *Andreaeana* because the former generic name had previously been employed for a genus of mosses. This correction was published as a supplement in connection with their earlier report.

In 1928 van Beyma Thoe Kingma (1928) concluded from examination of cultures isolated by Palm and Jochems that the mold was identical with *Oospora nicotianae* Pezz. et Sacc.

In 1913 Sumstine (1913), in a monographic study of fungi of this type, created the genus *Torulopsis* and properly placed in it the stack mold fungus, giving it the name *Torulopsis nicotianae* (Pezz. et Sacc.) Sumstine.

Palm and Jochems (1923) determined that tobacco saturated with moisture and maintained at a temperature of 33° C. provides optimum conditions for the development of stack mold. The humidity and temperature within the stack, therefore, determine the extent of the damage. They recommend that the stacks be kept air-dry and that excess moisture within the stacks be avoided by proper stacking. If the stacks become moldy, they should be pulled down and rearranged. Tobacco stored at 10° to 20° C., they found, would not become affected with this decay.

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MUSTINESS OR WHITE MOLD

Air-cured tobacco that is stored in cool, badly ventilated rooms or that is exposed to these conditions in

FIG. 111. Mustiness or white mold on a hand of air-cured tobacco. The peculiar odor and whitish coating are characteristic of this decay. (From James Johnson.)

transit, on shipboard, to market is oftentimes found to have a musty odor. The leaves, if examined, might be found to be quite all right, or there might be whitish patches on them. This condition has been investigated by Gandrup (1923) in connection with tobacco grown in Java. He found that "krossok" tobacco, that cured by the Javanese, was especially prone to be musty on arrival in Europe. This tobacco proved to have been too moist when packed, and to have been kept in poorly ventilated storage. He found that if it were dried properly before storage and kept dry subsequently in well-ventilated rooms, no mustiness developed.

In his studies to determine the proximiate cause of mustiness he learned that it was not associated with the various molds commonly found on cured tobacco leaves, nor with bacteria. His isolations yielded several species of *Actinomyces* which, when used to inoculate normal cured leaves, resulted in the development of the same odor as occurs in naturally contaminated leaves.

Species of *Actinomyces* are commonly present in soils and are productive of decomposition of organic matter in soils. Some few species are associated with diseases of the underground parts of plants, as the potato scab disease, or with mycoses in animals. Gandrup (1923) is of the opinion that *Actinomyces* from the soil in the tobacco fields is splashed upon the leaves by rains, and that it remains inactive upon the surface of the leaves except when suitable temperature and moisture conditions obtain in storage.

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Spoilage of Flue-Cured Stored Tobacco

Flue-cured tobacco, as sold on the warehouse floor, has a moisture content above that which is safe for the ageing process. It must, therefore, be redried and its moisture content restored to 8 to 9 per cent of its dry

weight before being packed into hogsheads and placed in storage.

The writer has examined several commercial grades of flue-cured tobacco for its flora of bacteria and molds. These examinations were made at intervals from the time the tobacco came from the redrying machines until approximately two years later, when it was aged and ready to be used in the manufacture of cigarettes. The results of these examinations show that flue-cured tobacco has a "normal flora" throughout this entire period. There is little seasonal variation in the kind and number of organisms present, apparently due mainly to the uniformly low moisture content. This lack of moisture inhibits the growth of microörganisms, for when moisture is supplied spoilage readily occurs. There is a tendency for all of the commercial grades to have the same kinds of molds and bacteria and for the numbers to decrease with the duration of storage. Not all organisms have perished, however, after several years in storage.

Such cosmopolitan molds as species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, and *Alternaria* are universally present. Occasional samples yield *Actinomyces*. *Bacillus subtilis* and *Bacillus mycoides* are among the species of bacteria most commonly present.

Spoilage of flue-cured tobacco is not a serious problem, as might be anticipated from the fact that it is relatively so dry. In cases where it occurs, spoilage may be checked by elevating the temperature and thus increasing the relative ability of the air to hold moisture.

Spoilage of Manufactured Tobacco

This phase of the decay of tobacco has received attention at the hands of True (1914) and of Wollenweber (1928).

True's investigations of moldiness of cigars revealed that the paste commonly used contained the conidia of such species of fungi as *Rhizopus nigricans* Ehrb., *Mucor racemosus* var. *brunneus* Morini, *Penicillium* sp., and *Aspergillus candidus* Link. The leaves used as wrappers

were contaminated with *Aspergillus subgriseus* Peck, *Sterigmatocystis castanea* Patters., *Cladosporium herbarum* Link, and *Penicillium* sp. His examinations of molded cigars showed that *Aspergillus candidus* was quite universally present and appeared to be responsible for most of the spoilage. Since this mold did not occur upon the leaves used in manufacturing the cigars but in the paste, he recommended that cigar manufacturers use boric acid as a disinfectant in the paste. When this was done, moldiness of cigars was practically eliminated.

Wollenweber found that *Penicillium glaucum* Link and *Aspergillus glaucus* (L.) Link occur most commonly on cigarettes stored in warm, damp situations, and that they are responsible for the impaired aroma and taste of cigarettes. This condition is encountered in shipments of cigarettes sent into the tropics, and is otherwise of infrequent occurrence.

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